PCT/GB99/03558

The Patent Office

INVESTOR IN PEOPLE

GB 99/3558

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

4

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gerry

Dated 5 November 1999

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patents Form 1/77

Act 1977
(Rule 16)

THE PATENT OFFICE
C
28 MAY 1999
LONDON

The Patent Office

01JUN99 E451097-1 D02094
P01/7700 0.00 - 9912641.9

Request for grant of a patent

1/77

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

28 MAY 1999

1.	Your reference	SJK/SH/VAL/33838
2.	Patent application number	**9912641.9**
3.	Full name, address and post code of the or each applicant	Ashby Scientific Ltd. Unit 11, Atlas Court, Atlas Road Hermitage Industrial Estate, Coalville, Leicestershire LE67 3FL United Kingdom
	Patents ADP number	6736409002
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
4.	Title of the invention	Textured and Porous Silicone Rubber
5.	Name of your agent	VENNER, SHIPLEY & CO
	"Address for service" in the United Kingdom to which all correspondence should be sent	20 LITTLE BRITAIN LONDON EC1A 7DH
	Patents ADP	1669004

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and the or each application number	Country	Priority application number	Date of filing
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and filing date of the earlier application		Number of earlier application	Date of Filing
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request?	YES		

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	34
Claim(s)	17
Abstract	1
Drawing(s)	8 + 8

10. If you are also filing any of the following state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents	

11. I/We request the grant of a patent on the basis of this application.

Signature: Venner, Shipley & Co.

Date: 28 May 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

Dr. Stephen Killin
0171-600 4212

Textured and Porous Silicone Rubber

Description

The present invention relates to methods for manufacturing silicone rubber that is adapted to promote cell adhesion and growth, in particular, providing silicone rubber with a modified surface or structure for enhanced cell attachment. The silicone rubber is particularly well suited to a variety of tissue culture and medical applications.

Silicones surpass other elastomers in many performance categories because of their rigid silicone-oxygen chemical structure. The process of vulcanisation transforms this structure, allowing the silicone-oxygen polymer to become an elastic rubber. Silicone rubbers are stable throughout a temperature range of -46°C to 232°C. They are odourless, tasteless and do not support bacterial growth. Silicones also do not stain or corrode with other materials. Most importantly, silicone rubber is not physically or chemically degraded or altered by contact with body fluids, and is not toxic or allergenic to human tissue and will not excite an inflammatory or foreign body reaction. Silicone material can be formulated and tested for full bio-compatibility, complying with guidelines for medical products. A further and particularly important advantage of silicone rubber is that it has the highest oxygen permeability of known polymers.

Forming textured and porous silicone rubber allows all of the advantageous properties of silicone rubber to be exploited and enhanced. For example, a textured surface will not only greatly increase the available surface area for cell attachment, but will also encourage the cell attachment. Furthermore, the increased surface area will increase the oxygen permeating through the silicone, enhancing the metabolic activity of the cells attached thereto. These advantages are very important in the proposed uses of the textured and porous silicone rubber discussed below.

According to a first embodiment of the invention method is provided of making a silicone rubber having a structure adapted for growth of cells or living tissue, which comprises contacting a silicone rubber precursor with a biologically-acceptable sacrificial filler, curing the resultant mixture and removing the sacrificial filler to form a structured silicone rubber.

In a preferred embodiment, the silicone rubber precursor used can be cured or vulcanised at room temperature. This obviates the need to expose the mixture to elevated temperatures. This is particularly useful because some sacrificial fillers become unstable and decompose at elevated temperatures, making it difficult to control the form of the structured silicone rubber.

In a further preferred embodiment, the biologically-acceptable sacrificial filler is biocompatible, such that it is innately non-toxic and does not leave a toxic residue. This is of particular significance where the structured silicone rubber is intended for use in tissue culture and medical applications.

There are a number of further factors to be considered in the selection of the sacrificial filler to be used in the method according to the first aspect of the invention.

The sacrificial filler should not react with the silicone rubber to be used, either as the precursor or as cured silicone rubber.

The filler should also be soluble in order to facilitate its removal. The solvent used to dissolve the material should also not react with the silicone rubber.

If the silicone rubber layer is to be cured at elevated temperatures, then sacrificial filler that is stable at the curing temperatures should be used. The reactivity of the sacrificial filler at elevated temperatures is also a consideration. Material that either melts or decomposes at high temperatures may not be suitable.

Finally, the sacrificial filler used should be relatively inexpensive and readily available.

Where a specific crater size is required, in a preferred embodiment the sacrificial filler is ground and, preferably, classified, prior to contacting the silicone rubber precursor. This will allow the form of the structured silicone rubber to be more accurately controlled. One method for grinding the sacrificial filler is to wet-mill it, prior to mixing with the silicone rubber precursor.

Desirably, the sacrificial filler is milled to a particle size of 0.01-10 μm, preferably 0.05-1 μm, and most preferably 0.1-0.4 μm.

Where the sacrificial filler is an inorganic salt, in another preferred embodiment, it may be milled in an organic solvent.

In an embodiment, the sacrificial filler is granular and, preferably, crystalline. Alternatively, the sacrificial filler used may be amorphous.

Preferably, the sacrificial filler is an inorganic salt selected from the group consisting of metal halides, metal carbonates and metal bicarbonates. More specifically, it is selected from the group consisting of lithium bicarbonate, sodium bicarbonate, potassium bicarbonate, lithium chloride, sodium chloride and potassium chloride.

In the most preferred embodiment, the sacrificial filler used is either sodium bicarbonate or sodium chloride, preferably food grade sodium bicarbonate or sodium chloride.

The sodium bicarbonate or sodium chloride may be wet-milled under xylene.

In a preferred embodiment of the first aspect of the invention, the sacrificial filler is removed by dissolution, preferably in an aqueous solvent. The sacrificial filler should be chosen so that it does not cause swelling of the silicone rubber when removed using an aqueous solvent.

In a preferred embodiment of the first aspect of the invention, the free –OH groups of the silicone rubber are chemically modified, so as to enhance cell adherence.

In another preferred embodiment, the surface of the silicone rubber is charged by bombardment with electrons.

In a preferred embodiment, the silicone rubber precursor includes at least one additive that is not removed with the sacrificial filler and serves to impart desired physical properties on the rubber. The additive may be a metal powder or carbon black and serves to render the

silicone rubber electrically conductive. Alternatively the additive may be stainless steel powder, to increase the density of the silicone rubber. Another preferred additive is iron oxide. The additive may also be an inert substance, such as glass, to render the silicone rubber mechanically rigid.

According to a second aspect of the present invention, a method is provided wherein a surface of the silicone rubber precursor is contacted with the sacrificial filler, so as to form a structured silicone rubber having a textured surface.

The textured surface of the silicone rubber facilitates attachment of adherent cells. It also provides an increased number of sites for attachment of cells relative to an untextured surface.

In a preferred embodiment of the second aspect of the invention, a method of making the textured silicone rubber comprises forming a coating of a silicone rubber precursor on a substrate, contacting a surface of the coating with a biologically-acceptable sacrificial filler, curing the resultant mixture and removing the sacrificial filler to form a textured silicone rubber.

In one version of this method, the surface of the coating is contacted with the sacrificial filler under pressure, such that the sacrificial filler is substantially completely embedded in the coating.

Preferably, this results in the sacrificial filler being embedded to a depth of 0.1-1.0 mm, preferably 0.1-0.5 mm, and most preferably 0.1-0.25 mm.

In an alternative version of the method, the sacrificial filler is scattered or sprinkled over the surface of the coating, such that the sacrificial filler is only partially embedded in the surface.

Preferably, the resultant textured surface is micro-cupulated, the micro-cupules having a depth of less than 1 mm, preferably a depth of 0.5-0.1 mm. Desirably, the micro-cupules

measure less than 2 mm across, preferably less than 1 mm across, and, most preferably, less than 0.5 mm across.

In order to obtain the desired micro-cupulated surface structure, the material used in the method described must be selected carefully.

Silicone rubbers are available with different physical properties, both in the uncured and cured state. The method of cure can also differ widely. Thus, the silicone rubber can affect the manufacturing process and selection of a suitable silicone rubber is therefore very important.

When using the manufacturing process according to the second aspect of the present invention, a silicone rubber should be selected with consideration to the manner in which the mixture is to be applied to the substrate, the conditions required for curing, and the desired properties of the end product. The uncured silicone rubber should have an appropriate viscosity for the method of application to the substrate, and should retain its general form once the sacrificial filler has adhered to its surface. The conditions for curing must be compatible with both the substrate to which the uncured silicone rubber is applied and the sacrificial filler that adheres to the surface. Finally, the quality of the silicone used should also be selected in light of the intended application of the final product.

In a preferred embodiment, silicone rubber paint RTV 118 (General Electric Co., Connecticut, USA) is used.

In order to assist adhesion of the silicone rubber layer to some materials, it may be necessary to apply a conventional adhesive, such as a mineral spirit based primer, prior to deposition of the silicone layer. In a preferred embodiment, the primer used is silicone rubber primer SS 4155 (General Electric Co., Connecticut, USA).

The cratered or micro-cupulated silicone rubber surface formed using the above manufacturing method may be applied to any substrate.

The textured surface has been found to produce a greatly increased yield when used in tissue culture processes. The textured surface provides increased surface area for cell attachment, and encouraging cell attachment. The increased surface area also enhances oxygen supply to the surface. Thus, the above described textured silicone layer may be used in a variety of devices, in particular those where cell attachment is important.

According to a third aspect of the present invention, the sacrificial filler is dispersed throughout the silicone rubber precursor, and the structured silicone rubber is substantially porous.

The pores of the silicone rubber provide sites of attachment for cells or tissues, so that the cells or tissues may be substantially trapped within the resultant structure.

The method also creates a system of pores and channels throughout the structure. This system of pores can act as a capillary system, increasing oxygen and nutrient supply to the surface of the structure

In a preferred embodiment, the method of making a porous silicone rubber comprises mixing the biologically-acceptable sacrificial filler with the silicone rubber precursor, curing the resultant mixture at a temperature below 180°C, and removing the sacrificial filler, to form a porous silicone rubber.

The resultant mixture is shaped prior to curing, preferably by moulding or extrusion.

In a preferred embodiment, the pores formed are 1 μm-0.5 mm, preferably 10 μm to 0.2 mm, and most preferably 50 to 150 μm in diameter.

Desirable, the porous silicone rubber is cut to a desired size or shape.

Silicone rubbers frequently contain innate filler that is added to produce desired viscosity, strength and other physical properties. The amount of sacrificial filler that can be mixed into the rubber and therefore the extent of the porosity achieved is in direct proportion to the quantity of innate filler (such as fumed glass) already present in the rubber. Thus, a low

viscosity rubber with little innate filler can be given a greater packing density of sacrificial filler than a high viscosity which has high levels of innate filler to give it a thicker consistency.

The viscosity of the rubber is also key when considering the manner in which the mixture is to be manipulated to give the end product. For example, if the mixture is to be extruded, then a low viscosity rubber, although able to hold the maximum filler, would not be suitable for two reasons. Firstly, say separation would occur if small cross sections were to be required and secondly, due to the low viscosity, slumping of the mixture and hence distorted shapes would arise. However, if the material were to be spread into a sheet or moulded, then the low viscosity would prevail as for the same amount of filler it would be more easily manipulated using these techniques.

The green strength, that is the strength of the uncured precursor mixture, is also a factor for consideration. Low viscosity rubber, when packed with sacrificial filler, exhibits very poor green strength and is hence undesirable for extrusion. The ideal would be a very high viscosity rubber, however so little sacrificial filler can be mixed into these materials that they are not a practical option. Therefore, a rubber somewhere between the two must be found so that enough innate filler may be included to maintain green strength but little enough to be able to pack in sufficient sacrificial filler.

The cure regime of the rubber must also be taken into account. Where a rapid cure is required, for example to maintain the geometry of an extrusion, then heat cure systems are required. However, these systems must be tailored such that the heat process does not have a deleterious effect on the filler. It may also be necessary to use room temperature curing systems if the material needs to be bound to an additional substrate that is not able to withstand elevated temperature such as thermoplastic plastics.

The physical properties of the cured rubber must also be considered. Where tensile strength is an issue, such as in the formation of tubes or sheets, then a rubber with high tensile properties must be used. Such rubbers tend, however, to be of higher viscosity and contain large amounts if innate filler. Hence a compromise must be found. Where tensile strength is less of an issue, a low viscosity rubber may be used, especially if there is no

requirement for extrusion. Finally, the actual grade of silicone is worthy of note. The final application of the material will determine the quality of the rubber used. For medical and implantable applications, an unrestricted grade of material should be used, with the other extremes being the use of industrial grade silicones for applications such as waste water treatment.

In some instances, it is desirable to add additives to the mixture to achieve certain characteristics such as a required density, magnetic properties and the like. In the majority of cases, such compounds would be in a powder form and the considerations needed to choose these materials would be similar to those for the sacrificial filler. For example, if the material was required to have an increased density, then a high mass powder would be added in small quantities to make these adjustments and the choice of powder would follow criteria such as reactivity, toxicity and economics etc.

Experiments using the above described methods of manufacturing three dimensional porous silicone rubber showed that the use of sodium chloride as the sacrificial filler caused the silicone to swell. Sodium bicarbonate was found to satisfy the above discussed criteria for sacrificial fillers although it decomposes and therefore "blows" the material at temperatures above approximately 180°C. It has therefore been necessary to adapt the manufacturing process to avoid temperatures above 180°C, for example by selecting silicone rubbers, which cure at lower temperatures. Many of the alternative sacrificial fillers are toxic, leave toxic residues when dissolved, or are problematic at moderate temperatures required for working with silicone rubber.

The silicone rubbers formed using the methods in accordance with the first, second and third aspects of the inventions have properties that make them particularly well suited use in a biomedical device or apparatus.

According to a fourth aspect of the present invention, there is provided a culture chamber for use in a method of culturing microbiological material, which comprises at least one gas-permeable wall or portion of a wall, and a textured interior growth surface arranged for contact with the microbiological material being cultured.

The *in vitro* culture of cells is well established in the field of biotechnology, the term cell culture being interpreted to mean both growth and maintenance of the cells.

In a preferred embodiment, the gas-permeable wall and the textured interior growth surface are each formed from an organic polymer. These features may also be formed of the same organic polymer.

The at least one gas-permeable wall or portion of a wall of the culture chamber may also provide the textured interior growth surface.

In a preferred embodiment, the textured interior growth surface is obtained or obtainable by a method according to the second aspect of the present invention.

Preferably, the gas-permeable wall is a silicone rubber membrane.

In a preferred embodiment, the culture chamber has at least one port extending between the interior and the exterior of the chamber. Preferably, there will be an inlet and an outlet port. An additional septum port may also be provided.

In a preferred embodiment, the culture chamber is in the form of a flexible bag or envelope.

A variety of different apparatus is known for the culture of cells *in vitro*. In recent years, flexible culture bags have become increasingly popular, offering a number of advantages over the traditional cell culture apparatus, such as multi-well plates, flasks, roller bottles and spinner flasks. For example, culture bags represent closed systems, reducing the risk of contamination. They also take up less storage and incubator space. The bags are relatively cheap, making them effectively disposable and reducing the need to sterilise them for re-use.

Aeration of the culture is essential in order to provide the cells with oxygen necessary for growth. In the past, methods such as sparging, surface aeration, medium perfusion have

been used to increase oxygen availability. However, such methods can cause cellular damage, severely limiting the efficiency of cell culture.

Silicone has the highest oxygen permeability of known polymers, and silicone rubber tubing or membranes making it well suited for use in cell culture, where it provides improved diffusion of oxygen to the cells. Silicone rubber not only provides gas permeability (including oxygen and carbon dioxide) but also vapour transmission, structural integrity, resilience and temperature resistance, all of which are desirable in cell and tissue culture.

International application no. PCT/US96/20050 (Avecor Cardiovascular Inc.) discloses a cell culture bag formed from a plurality of thin, spaced, gas-permeable silicone membranes. The gas exchange rate provided by the silicone bag is significantly higher than most conventional culture bags. However, whilst such bags have shown higher cell density and cell viability, this is still limited by the surface area of the bag.

To date, the interior surface of culture bags have been smooth. Such smooth growth substrates provide limited surface area and cell attachment features. Thus, the known culture bags do not provide growth substrates for efficient cell culture of anchorage-dependent cells. Furthermore, certain "problem" cell types are unable to attach to the smooth interior surface of the known culture bags.

An elaborate (and seemingly expensive) method of increasing the surface area for cell adhesion is described in US patent no. 4,937,194 (Baxter International Inc.), wherein the flexible bag contains material of a cellular structure, such as a honeycomb type structure with hexagonal channels passing through it, serving as adherent sites for the cells that are cultured. The use of a microcarrier, such as small glass spheres or sodium alginate, is also proposed to increase the surface area for cell adherence inside the culture bag.

The cell culture bag according to the fourth aspect of the present invention provides an increased growth substrate surface area for cell attachment. The bag of the invention will also provide a growth substrate that will assist cell attachment. Importantly, the bag structure is simple and cheap to manufacture.

In a preferred embodiment, the bag is made from at least one silicone rubber sheet that is coated with a silicone rubber layer having a rough or uneven micro-cupulated growth surface exhibiting a plurality of craters or crater-like depressions.

Preferably, room-temperature vulcanising silicone rubber is used and the preferred sacrificial filler for use in producing the textured surface is sodium chloride

The micro-cupulated surface significantly increases the surface area for cell attachment, increasing the efficiency of the cell culture.

The micro-cupulated surface also assists attachment and growth of certain "problem" cell types. "Problem" cell types include, for instance, stromal cells necessary for stem cell expansion processes. Stromal cells originating from bone require a textured surface on which to grow if their proliferation is to be optimised.

The culture bag of the present invention preferably also includes one or more ports, extending between the bag interior and bag exterior. Such ports may be used for introducing nutrient medium, taking samples, adding further ingredients, etc. The ports should have valves, locks or the like, to avoid contamination of the bag interior.

In a preferred embodiment, the culture bag is provided with an inlet and an outlet port with luer locks, and a septum port for taking samples or introducing substances into the bag. The ports are desirably positioned between the sealed edges of the culture bag.

The rough interior coating surface of the culture chamber makes the wall opaque. Thus, in a further embodiment, there is provided an area of the membrane to which the rough or uneven layer is not applied, this area therefore providing a transparent window, allowing one to see the inside of the culture chamber.

In further embodiments, the culture chamber may include a valve means, allowing the release of gases that build up during cell growth and form an air bubble inside the bag. The presence of a bubble within the chamber will prevent colonisation on the surface area

adjacent the bubble because said surface will not be in contact with the culture medium. A valve in the culture chamber wall will minimise the size of the gas bubble, thereby maintaining a large surface area in contact with the nutrient medium and available for cell attachment. Thus, almost full colonisation on the interior chamber surface is possible, increasing the efficiency of the culture chamber of the present application.

Desirably, the valve comprises a filter means, allowing gasses to diffuse out of the chamber but preventing microbial contamination. In a preferred embodiment, the valve means comprises one or more layers of a hydrophobic material, such as a hydrophobic PTFE membrane having a thickness of 0.25mm and a porosity of 0.2 microns.

The growth surface of the culture chamber according to the present invention may be treated to further enhance cell adhesion, for example by charging the surface by bombardment with electrons. It is also possible to modify the free -OH groups of the silicone rubber surface to encourage attachment of various chemical moieties. Alternatively, cell attachment to the growth surface of the culture chamber may be promoted by adapting the size of the micro-cupules or depressions to the specific requirements of the cells to be cultured.

In a preferred embodiment, a culture chamber further comprises a second chamber separated from the first chamber by means of a semi-permeable membrane. The second chamber has an access means separate from that of the first chamber.

According to a fifth aspect of the invention, an apparatus is provided comprising a plurality of culture chambers of the fourth aspect, for use in a method of culturing microbiological material.

The inlets of the culture chambers are interconnected and the outlets of the culture chambers are interconnected.

In a preferred embodiment, the apparatus has a further chamber having a semi-permeable wall is positioned within each culture chamber, each semi-permeable chamber having an

inlet that is interconnected with the inlet of the other semi-permeable chambers and having an outlet that is interconnected with the outlet of the other semi-permeable chambers.

In a preferred method, anchorage-dependent stromal cells are grown on the textured surface of the culture chamber(s), and then anchorage-independent stem cells are inoculated into the culture chamber(s), to allow proliferation of the stem cells.

Preferably, the apparatus is used as a bioreactor.

The bioreactor is particularly applicable to the bio-processing of liquors containing particulate matter, such as blood cells or cell debris.

Bio-reactors according to the prior art are normally closed systems, and have such have the disadvantages of relatively low productivity and efficiency. A particular drawback is the limited volume of oxygen available for the reaction in such closed systems. They are, moreover, not normally suitable for the processing of liquors containing particulate matter, such as whole blood.

The bio-reactor according to the present invention does not suffer from the above problems, comprising oxygen permeable walls, and textured surface of silicone rubber to assist the growth process of the bio-substances. The product is subsequently generated in a continuous process, by a passage of liquid nutrient medium over the bio-substances.

In a preferred embodiment, a method of carrying out a bio-processing operation in a culture chamber or an apparatus comprises attaching cells for performing the bio-processing function to the textured surface of the culture chamber (s), introducing liquor to be processed into the culture chamber(s) via an inlet and collecting the processed liquor at an outlet from the culture chamber(s).

Preferably, the spent medium including cellular by-products is removed from the culture chamber(s), and fresh nutrient medium is passed through a semi-permeable chamber(s) located within the culture chamber(s), so to allow fresh medium to diffuse through the semi-permeable membrane into the culture chamber(s).

Advantageously, the nutrient medium is passed through the semi-permeable chamber in the opposite direction to that in which the liquor or spent medium is passed through the culture chamber. The nutrient medium may be recycled.

For use of one of the preferred embodiments, the apparatus is filled with the liquid medium, which has first been inoculated with the desired cell line. The assembly of reactor tubes may then be arranged to be rotated or agitated, for example, using machinery such as that employed for conventional roller bottle reactors. Rotation is continued until cell confluence is obtained, as evidenced by the levelling of the rate of glucose uptake. The inner surfaces of the reactor tubes are therefore extensively coated with the cells as this stage. Rotation may, where appropriate, be interrupted for replacement of the medium in the reactor.

The reactor tubes are then removed from the rollers and connected to a suitable media reservoir. A continuous stream of liquid nutrient medium is arranged to pass through the reactor envelopes, the product being harvested at the outlet. During this procedure it is desirable to provide an airflow over the reactor, to assist oxygenation.

According to a further preferred embodiment, the apparatus is especially adapted for the bio-processing of liquors containing particulate matter, such as blood cells or cell debris. The continuous flow system according to the invention is especially applicable to the processing of whole blood, as in an artificial extra corporeal organ, for example substituting or supporting the functions of the human liver. Notably, the system obviates the need of separating the particulate matter prior to processing and then having to reunite the constituents.

It is also anticipated that the chambers and apparatus may have other medical applications such as the expansion of other primary cell types or acting as an *ex vivo* model for drug metabolism if colonised with hepatocytes and the like.

For these purposes, apparatus is provided comprising the above described culture chambers, within which are positioned semi-permeable chambers, for example, of cellulose

acetate. These semi-permeable chambers are arranged to be separately connected to common inlets and outlets at their respective ends. The bio-processing operation then involves the following procedures. Firstly, the cells grown to perform the bio-processing function are attached to the textured surface of the culture chamber, as described above. The medium is then removed from said culture chambers. The nutrient medium is then passed through the semi-permeable chambers, introduced from a reservoir through the inlet at one end of the semi-permeable chambers, issuing at the outlet on the opposing end. If desired, the medium may be recycled from the outlet, to return again to the inlet of the chambers.

The liquor to be processed, for example blood, is then arranged to flow through the culture chambers, the textured interior surface of which are now coated with cells. The liquor is introduced for this purpose at the inlet of the culture chambers, formerly serving at the medium inlet, and issuing at the outlet on the opposing end. The liquor is preferably passed through the culture chambers in opposing direction to that of the nutrient medium. During this procedure, nutrients from the medium pass through the semi-permeable chambers, traversing the stream of liquor, to feed the cells adhering to the coating of the culture chambers. At the same time they also perform the function of cleansing the liquor of waste materials such as ammonia and urea, etc. The treated liquor is finally collected at the outlet of the culture chambers.

The productivity and efficiency of the growth process, especially in the case of anchorage dependent cells, can be substantially enhanced using the above described bio-reactors, as compared with conventional reaction vessels. Generally, the systems described in the prior art do not utilise oxygen permeable containers, and as such cannot sustain the cell growth process in the manner made possible by the bio-reactors in accordance with the present invention.

According to a sixth aspect of the present invention, a well for use in a method of culturing microbiological material is provided, having at least one wall defining the well, at least a portion of the wall being gas-permeable to enhance oxygen supply to the well, and at least a portion of the interior surface of the wall being textured to increase surface area and to enhance cell adherence.

Preferably, the gas-permeable portion of the wall and the textured portion of the wall are positioned at the base of the well.

In a preferred embodiment the gas-permeable portion of the wall comprises a gas-permeable membrane, preferably formed of silicone rubber.

The membrane preferably has a textured surface facing the interior of the well.

In a preferred embodiment, the textured surface has crater-like depressions or micro-cupules. Desirably, this textured surface is made by a method according to the second aspect of the present invention.

A microtitre plate having at least one well according to this aspect of the invention is envisaged.

The wells according to this aspect of the invention enhance both the quantity of cells that can be grown in a microtitre well of a given size, and their metabolic activity.

Currently, as microtitre wells are increasingly minimised, cells grown in them for drug metabolism studies are decreased in number by the simple decrease in available growth surface. The cells are also starved of oxygen due to the decrease in gassing surface to volume ratio. The plate with the silicone membrane base would eleviate this problem by firstly increasing the available surface area with the textured surface and secondly allowing the cells to be gassed from below the second membrane.

According to a seventh aspect of the present invention, an implant device comprising a cell support structure having a coating with a textured surface, to promote anchorage of the implant by cell attachment and ingrowth by surrounding tissue upon implant.

Preferably, the textured surface has crater-like depressions or micro-cupules.

In a preferred embodiment, the coating comprises textured silicone rubber, preferably manufactured according to the method in the second aspect of the present invention.

Such implant devices may be used for a heart valve, a sternum implant, or a reconstructed calf ligament.

The textured surface on the implants acts as an anchor for tissue ingrowth. Thus, the textured implant can help to either prevent migration of larger implants or to promote a secure bond where the interface with the implant and the surrounding tissue is critical.

It has been shown that the textured surface has the further advantage of reducing the formation of capsule-type scar tissue when implanted.

According to a eighth aspect of the present invention, a substrate for growth of skin grafts *in vitro* is provided, comprising a flexible membrane having a textured surface.

A major problem associated with the growth of skin *ex vivo* is that when it is grown on a solid surface, the skin tends to be brittle and does not have the opportunity to "learn" to be flexible. In addition, the undersurface of the skin tends to be smooth and scar-like, which makes it difficult for the skin graft to take.

The flexible membrane used in this aspect of the present invnetion prevents the skin graft from becoming brittle, whilst the textured surface increases the surface area for cell adhesion, promotes cell adhesion and gives the skin a rough surface to enhance "taking" of the graft on transplant.

Preferably, the flexible membrane is gas-permeable, comprising a material such as silicone rubber.

In a preferred embodiment, the textured surface has crater-like depressions or micro-cupules. Preferably, the textured surface is manufactured according to the method of the second aspect of the invention.

The textured surface not only offers the greater surface area for cell growth, but also a degree of ingrowth into the silicone in small areas, so that upon removal from the growth surface, the skin undersurface will be textured, assisting the taking process of the graft. In addition, of course, the oxygen permeability of the silicone would assist in promoting the metabolic activity of the growing graft.

In accordance with a ninth aspect of the present invention, a tissue support structure is provided for use in a method of culturing tissue or cellular agglomerates, which comprises a biocompatible material having an internal system of pores, the pores promoting cell attachment and anchorage and oxygen supply to the tissue.

Microparticles of *ex vivo* grown organs have many applications in the drug development industry. However, known devices acting as support substrates for tissue grown *ex vivo* are severely limited as to the size of the tissue agglomerates that may be grow.

The need to provide oxygen and nutrients to the centre of a three dimensional tissue mass has been recognised in the prior art and there has been addressed in a number of different ways, all of which involve complex, and no doubt expensive, support structures with specific structural features for gas and nutrient supply.

The tissue support structures according to this aspect of the invention have a system of pores and channels within the porous structure that is capable of mimicking a capillary system, delivering oxygen directly to the centre of the tissue growing on the silicone structure. This allows much larger silicone agglomerates to be formed whilst avoiding necrosis and apotosis.

In a preferred embodiment of this aspect of the invention, the porous material is provided with small, fine bore tubes.

In another preferred embodiment, the shape of the porous material may be adapted so as to engineer the shape of the resultant tissue.

Preferably, the porous material comprises porous silicone rubber, for example, as is made according to the method of the third aspect of the invention.

An apparatus for culturing tissue or cellular agglomerates is also provided, comprising a tissue support structure and a gas-permeable membrane, to enhance oxygen supply to the system of pores and channels within the porous material, and therefore to the tissue.

Preferably, the gas-permeable membrane is attached to the porous material. The gas-permeable membrane may be silicone rubber.

Advantageously, the porous material is attached to the gas-permeable membrane using a gas-permeable adhesive, such as a silicone rubber adhesive.

In a preferred embodiment the plurality of tissue support structures are arranged in close proximity to one another, so as to allow fusion between tissue or cell masses growing on each structure, to create larger tissue or cellular agglomerates.

It is envisaged that tissue grown on this type of structure could reach macro dimensions being fed with oxygen via diffusion through the solid threads from the tubes through which oxygen would be passed.

Preferably, the support structure is in the form of a pillar, the dimensions of which are approximately 0.25mm x 2mm.

According to a tenth aspect of the present invention, an artificial implant is provided, formed from a material having an internal system of pores, the pores promoting cell attachment and anchorage and oxygen supply to the cells on the implant surface.

The textured surface of the silicone rubber enhances cell attachment and the pores throughout the structure allow a degree of ingrowth and anchorage of the cells, as well as a pathway for supply of oxygen to the cells on the surface.

In a preferred embodiment, the porous material comprises porous silicone rubber, preferably made according to the third aspect of the invention.

In one preferred embodiment, the artificial implant is for use as a cartilage implant. Preferably, the porous material is seeded *in vitro* with chondrocites, to form a layer of cartilage over the implant.

Such an implant may be used for replacing eroded joints, wherein the porous silicone structure has been moulded to conform to the shape of the bone, which it is to protect.

Such implants for use in the human body are of particular interest. In the prior art, cartilage for such purposes has been grown *in vitro* in a flat single layer on culture plates and is then placed over the eroded bone. The disadvantage of this conventional method is that the cartilage so grown is in a flat form and therefore does not readily accommodate to the contours of the bone to be protected.

In another preferred embodiment, the porous material of the cartilage implant could be moulded into the shape of a nasal bridge, or an ear.

This type of permanent synthetic bio-compatible implant offers support and a degree of permanent protection to the cartilage structure.

According to a eleventh aspect of the present invention, an artificial cornea is provided.

In a preferred embodiment, the artificial cornea comprises a lens surrounded by an annular skirt formed of the porous material.

The porous material allows infiltration of the skirt by surrounding tissue after implantation, to hold the cornea in place.

In a preferred embodiment, the lens is made from silicone rubber. The skirt may also be formed of porous silicone rubber, preferably made by a method according to the third aspect of the invention.

The artificial cornea is implanted under the skin, with the porous skirt promoting tissue ingrowth, to anchor the implant, giving a "living" cornea with an artificial lens in the centre.

Current processes involve the lengthy and disabling operation that removes a bone tooth composite from the patient's jaw that then has a lens glued into it. This device is then implanted under the skin.

According to an twelfth aspect of the present invention, a vascular graft is provided.

Vascular grafts can suffer from their base material having incompatible physical properties to those of the native tissue.

In a preferred embodiment, the vascular graft of the present invention comprises a hollow tube made from porous material, preferably porous silicone rubber.

The interior surface allows cell adhesion, and preferably endothelial cells are grown on the interior surface of the graft.

The exterior surface also allows cell adhesion, and preferably smooth muscle cells are grown on the exterior surface of the graft.

In a preferred embodiment, one or both surfaces of the graft are additionally roughened to enhance cell attachment, preferably by providing the graft with textured silicone rubber surface.

The elastic, compression and oxygen transport characteristics of three dimensional porous silicone rubber material closely mimics that of living tissue and hence problems such as stenosis may be overcome. Further advantages are that the grafts produce a lamina flow and not the turbulent flow associated with rigid synthetic grafts, hence minimising the problems of thrombosis. Due to the properties of silicone, grafts would be resealable,

which is advantageous for patients requiring repeated vascular access, for example patients suffering from renal disease and undergoing long term kidney dialysis.

According to a thirteenth aspect of the present invention, a cell implant means is provided comprising a porous material for retention of cells to be implanted, the pores promoting cell attachment and anchorage and oxygen supply to the cells, and a protective means to shield the cells from immune attack after implant.

In a preferred embodiment, the porous material comprises silicone rubber, such as is made by the method according to the third aspect of the invention.

The protective means desirably comprises a semi-permeable membrane forming an envelope around the porous material.

In a preferred embodiment, the cell implant means is for use as an endocrine implant. The porous material is seeded *in vitro* with endocrine cells. Preferably, the endocrine cells are islets of Langerhans cells.

The development of fully functioning endocrine implants, especially of the islets of Langerhans insulin secreting cells, has long been a target for clinical research. However, expanding islet cells has proved tricky, as it is difficult to make them proliferate in culture. Islet cells from foetal tissue have been proliferated but permanently lose function over time.

On implantation of the endocrine implant according to the present invention, the required hormone is released through the semi-permeable membrane, whilst this membrane also acts as a barrier to the body's own defences against the foreign cells. Regulation of the hormone released can occur naturally, as the feedback control molecules are able to pass through the semi-permeable membrane and communicate with the endocrine cells directly.

According to a fourteenth aspect of the present invention, a drug delivery system is provided, comprising a porous material whose pores have been impregnated or saturated with a drug for delivery.

Preferably, the drug delivery is suitable for implantation into a human or animal body.

In a preferred embodiment, the drug is present in admixture with at least one sustained release ingredient.

Desirably, the porous material comprises a porous silicone rubber. Preferably, the porous silicone rubber is made by a method according to the third aspect of the invention.

Certain drug groups diffuse readily through silicone rubber. Such drugs are incorporated into the three dimensional porous silicone rubber material which acts as a drug delivery system with certain advantages over the systems known in the prior art. Firstly, the porous nature of the material exposes large surface areas to bodily fluids for a vocatively small implant. Secondly, the synthetic nature of the material means that the system will not be rejected by the body when implanted. In addition, the material will not biodegrade as with many of the current devices, making it possible to explant the spent device for analysis and monitoring purposes if required.

According to a fifteenth aspect of the present invention, a filtration media comprising porous silicone rubber, for use in separations.

Preferably, the porous silicone rubber is made according to the third aspect of the invention.

In a preferred embodiment, the pores in the silicone rubber are of sub-micron size, preferably in the order of 0.1 – 0.5 μm.

The filtration media may be used in magnetic separation. In such a case, the porous silicone rubber preferably includes magnetic additives.

The filtration media may be used in expanded bed absorption. If so, the porous silicone rubber is preferably in particulate form.

The filtration media may be for use in static inline filtration. In such circumstances, the porous silicone rubber is preferably in the form of sheets or tubes.

A filtration media is also envisaged, wherein the porous silicone rubber is in the form or annular discs.

Preferably, porous silicone rubber with a sub-micron pore size is used. In its particulate form, the media is highly suited for use in the burgeoning market of expanded bed absorption technology. The material can be adjusted to have the appropriate density for this technology and due to its elastic nature can be used in whole, broth or continuous processes over protracted periods of time. After primary processing, the material can be made receptive to all common moieties used in affinity chromatography processes. In addition the material can also easily be made magnetic so that it can be easily separated from a whole broth system using magnetic separation.

According to a sixteenth aspect of the present invention, a cell cryopreservation system is provided comprising a porous material for absorbing cell culture into the internal system of pores and a container suitable for storage in liquid nitrogen.

In a preferred embodiment the porous material comprises porous silicone rubber, such as the porous silicone rubber is made by a method according to the third aspect of the invention.

The container of the cell cryopreservation system comprises releasable sealing means.

In an alternative embodiment, the container is a syringe-type plunger.

In such an embodiment, a number of cylindrical particles of porous silicone rubber may be in a tube fitted with a syringe type plunger. An operator could then suck up the required culture to saturate the porous silicone rubber particles and then store the device in liquid nitrogen. Upon retrieval, the operator then has a number of porous silicone rubber particles containing the same culture that can be used for several inoculums.

According to a seventeenth aspect of the present invention, an electrode is provided, comprising a porous material having electrically conductive particles dispersed therein.

In a preferred embodiment, the porous material comprises porous silicone rubber, for example the porous silicone rubber made by a method according to the third aspect of the invention.

Preferably, the conductive particles are metal or carbon powders.

In a preferred embodiment, the porosity of the electrode material promotes adherence of microorganisms to the electrode surface, preferably microorganisms that are capable of digesting waste. Such electrodes are suited to the treatment of sewage and similar applications.

An electrode system is also provided, comprising a plurality of electrodes immersed in a liquid electrolyte and connected to an electric circuit.

In use, as in conventional electrolytic systems, two electrodes (a cathode and an anode) are immersed in the liquid electrolyte, are connected to an electric circuit with a potential being applied between them. In special applications, electrolytic baths may comprise a plurality of electrodes.

The three dimensional porous silicone rubber electrodes have a number of advantageous features, including a large surface area and hence electrical capacity, robustness, inertness and resilience (aided by some degree of elasticity). These characteristics are particularly important in the relatively hostile chemical and physical environment of agitated liquid electrolytic cells. Furthermore, the fact that the porous silicone rubber material provides a favourable surface for the growth of micro-organisms makes such electrodes particularly suitable for special uses in water purification and sewage treatment applications.

Traditionally, such water treatments normally comprise the functions of converting (a) carbonaceous material to carbon dioxide and water, (b) nitrites to nitrates, and (c) nitrates to atmospheric nitrogen, all three functions relying on the actions of micro-organisms.

Among difficulties associated with conventional procedures are those of providing an adequate stream of oxygen through the sewage to maintain the micro-organism activity. This usually requires agitation of the liquid using mechanical stirrers, while passing a stream of oxygen through the sludge in the case of functions (a) and (b) and providing a safe reducing atmosphere for (c).

Using the three dimensional porous silicone rubber electrodes in the electrolysis process in, for example sewage treatment, advantages are achieved in respect of greater output efficiency, enhanced reliability with the absence moving parts in the system, as well as lower operating costs. By using the porous silicone rubber electrodes, an oxygen stream is applied at the anode to pass through the sewage, allowing micro-organisms to effect the reactions (a) and (b), while an enhanced level of hydrogen at the cathode aids the conversion by micro-organisms of (c).

According to an eighteenth aspect of the present invention, wound dressing is provided, comprising a first layer of a porous gel and a second layer of a carrier gel.

In a preferred embodiment, the porous gel layer comprises porous silicone rubber gel, preferably made by a method as defined in the third aspect of the invention.

The carrier gel layer may also comprise a silicone gel. Preferably, the carrier gel is applied to a supportive structure, preferably a Dacron® mesh.

In a preferred embodiment, the porous gel layer is infused with a drug for delivery to the wound. The drug is, for example, a growth-promoting drug or an antibiotic.

The wound dressing is designed to control scar formation by leaching low molecular weight silicones into the wound, a technology already employed in the field. This silicone gel wound dressing has the added advantage of increasing the contact area with fluids from the wound, thereby giving increased leaching and greater oxygen transport to the site, whilst maintaining asepsis. The drugs in infused into the porous structure leach into the wound over a prolonged period to aid the healing process.

According to an nineteenth aspect of the present invention, a clinical swab is provided, comprising a porous material, the pores increasing the surface area of the swab and promoting oxygen transport to the swab surface.

Preferably, the porous material comprises porous silicone rubber, for example the porous silicone rubber as made by a method of the third aspect of the present invention.

In a preferred embodiment, the porous material contains a radio-opaque additive, preferably barium sulphate. This would allow any lost swabs to be easily traced and then removed.

The porous material could also be infused with a drug.

The swab is preferably attached to the end of a stick, for example made of wood or plastic, as conventional swabs are, such as those comprising cotton wool.

The swab of the present invention has a number of advantages over the conventional swabs. The porous silicone rubber is oxygen permeable. The silicone is also non-linting, reducing the risk of debris being left after use. The silicone is also better attached to the stick than the cotton wool in conventional swabs. The silicone is furthermore chemically very stable and it will also allow microorganisms to adhere to the swab surface.

In order that the invention may be better understood, examples of the various aspects will now be described, by way of illustration only and with reference to the accompanying drawings, wherein:

Figures 1, 2 and 3 show successive steps of the manufacturing process in accordance with the second aspect of the present invention;
Figure 4 shows a cross-sectional view of three-dimensional porous silicone rubber in accordance with the third aspect of the present invention;
Figure 5 is a schematic plan view of a culture bag in accordance with the fourth aspect of the present invention;
Figure 6 is a schematic cross-sectional view of the culture bag of Figure 5;

Figure 7 is a cross-sectional view of a membrane wall of the culture bag of Figures 5 and 6; and

Figure 8 is an exploded view of the valve of the culture bag of Figures 5 and 7;

Figure 9 is a diagrammatic illustration of a bio-reactor apparatus according to the fifth aspect of the invention;

Figure 10 is a silicone rubber tube from the bio-reactor of Figure 9;

Figure 11 is a cross-sectional side view of a bio-reactor apparatus with dialysis tubes;

Figure 12 shows plan view of a mictrotitre plate according to the sixth aspect of the present invention;

Figure 13 shows a cross-sectional view of the plate of Figure 12 along line A-A';

Figure 14 shows a schematic artificial capillary system, in accordance with the ninth aspect of the present invention;

Figure 15 shows a part cross-sectional view of an endocrine implant according to the thirteenth aspect of the present invention.

Figures 1, 2 and 3 show successive steps of the manufacturing process in accordance with the second aspect of the present invention. As shown in Figure 1, the surface of a substrate 10 is firstly coated with a layer of uncured silicone rubber 11, for example by painting, spraying, etc.

Next, whilst the applied silicone layer is tacky and in liquid form, sacrificial filler is applied to the coating, to which they adhere, as shown in Figure 2. The sacrificial filler 12 can either be simply "sprinkled" onto the layer of uncured silicone rubber, or it can be applied under pressure. The material becomes partially embedded in the uncured silicone rubber. Any excess sacrificial filler that has not adhered to the uncured silicone layer is removed.

Then the silicone coating is allowed to cure. The conditions required for curing will be dependent upon the type of silicone used. Once the silicone layer has cured, the sacrificial filler which is still adhered thereto and partially embedded therein is removed by the dissolution in a solvent, thereby leaving a cratered or micro-cupulated surface structure as shown in Figure 3. The craters or micro-cupules 13 are permanently formed in the cured layer of silicone rubber.

Figure 4 shows the porous silicone rubber product of the manufacturing process according to the third aspect of the invention. As shown, the piece of three dimensional porous silicone rubber 70 has a textured exterior surface with craters 71, and pores 72 within the silicone, forming channels throughout the three dimensional structure.

In a specific example of the method according to the third aspect of the present invention, a porous silicone rubber is made using the silicone rubber GE Silicone's LIM 6070-D2 (part A & B) or McGhan NuSil's MED 4970 (part A & B). The sacrificial filler used is J. Astley & Sons Food Grade $NaHCO_3$ (sodium bicarbonate). Stainless steel powder is also added for a high density silicone product, namely MBC Metal Powders Ltd 316L SS fines 325 mesh.

The sodium bicarbonate is mixed with each of parts A and B of the silicone rubber separately, at a ratio of 3:1 w/w. The mixing is carried out using a conventional Z-blade mixer, although other mixer types may be used, or mixing may even be performed by hand.

If the density is to be increased, say for material destined for fluidised bed reactor, then the stainless steel powder is added to a level to give the desired density. Other high mass powders such as titanium oxide can be used.

Once mixed with the sodium bicarbonate, the parts A & B are stored separately in a cool place for further processing. The components must be kept apart as one contains the accelerator and the other the catalyst that will cause curing. If cross-contamination of the parts occurs, the material will start to cure.

When ready to cure the material into the required shape, parts A & B are mixed together on a two roll mill for 15 to 20 minutes to ensure complete mixing. Again other apparatus could be used.

The resultant mixture is then fed into a cold head extruder and extruded through a die of the appropriate shape. The resultant extrudate is picked up by a heat resistant conveyor and passed through a hot box set to such a temperature that the extrudate itself is heated to

175°C. This facilitates the cure of the material without allowing the sodium bicarbonate to decompose and hence "blow" the material.

Depending upon the geometry of the extrudate, it is passed through either a rotary cutter (for small cross sections) or a reciprocating cutter (for larger geometries) and chopped into the appropriate particulate shape.

This "preform" is the stored in a dry place until further processing is required. When required, the material is boiled in at least a five-times excess of pyrogen-free water for one hour. This process is repeated four or five times or until no further traces of sodium bicarbonate are evident, as indicated by the pH of the water.

The material is then finally rinsed in pyrogen-free water, bottled in an excess of the same and autoclaved to facilitate sterile storage. The material is now in a form ready for sale as a stand alone support matrix.

In another specific example of the method according to the third aspect of the present invention, the silicone rubber used is GE Silicone's RTV (room temperature vulcanising) 615 (part A & B). The sacrificial filler used is J. Astley & Sons Food Grade $NaHCO_3$ (sodium bicarbonate). For a high density silicone product, iron oxide (magnetic precipitate) from Fishers Scientific Products is used.

The sodium bicarbonate is wet milled under xylene using a Biaton bead mill to a particle size of 0.1 to 0.4μm. This range can be further narrowed by separation in a Malvern particle sizer. Using these methods, a whole range of particle sizes and distributions can be achieved.

The sodium bicarbonate is mixed with each of parts A and B of the silicone rubber separately, at a ratio of 3:1 w/w. The mixing is carried out using a conventional Z-blade mixer, although other mixer types may be used, or mixing may even be performed by hand.

If the density is to be increased, the iron oxide is added to a level to give the desired density. Other high mass powders such as titanium oxide can be used. Further weighting or magnetic moieties may also be mixed in, if required.

Once mixed with the sodium bicarbonate, the parts A & B are stored separately in a cool place for further processing.

When ready to cure the material into the required shape, parts A & B are mixed together on a two roll mill for 15 to 20 minutes to ensure complete mixing. Again other apparatus could be used.

The resultant mixture is then fed into a cold head extruder and extruded through the open scroll and collected as ingots on trays. The ingots are then cured at 150°C in a standard convection oven.

The ingots are then ground in a mill to the required size and can again be sized using a Malvern particle sizer if required.

This "preform" is the stored in a dry place until further processing is required. When required, the material is boiled in at least a five-times excess of pyrogen-free water for one hour. This process is repeated four or five times or until no further traces of sodium bicarbonate are evident, as indicated by the pH of the water.

The material is then finally rinsed in pyrogen-free water, bottled in an excess of the same and autoclaved to facilitate sterile storage. This product is biocompatible, it has pores in a very well defined size range and of an amorphous geometry.

The culture bag in accordance with the fourth aspect of the invention is shown in Figures 5 and 6. The culture bag 20 comprises two membranes 28 joined at their outer edges 27, each membrane having a textured (interior) surface 26. Inlet and outlet ports 23 extend between the inside and the outside of the bag, each port being provided with a valve 24. A degassing valve 22 is provided in the centre of one of the membranes, this membrane being uppermost when the bag is in use.

As shown in Figure 7, each bag membrane is prepared by covering the edges 27 of a smooth silicone rubber sheet 25 with a mask (not shown) and applying a layer of room-temperature vulcanising liquid silicone rubber to the exposed central portion of the sheet. Next, vacuum-dried salt is sprinkled over the layer of liquid silicone rubber so that it is uniformly covered. The liquid silicone rubber is then cured and the salt is washed out, producing a membrane 28 with a cratered or micro-cupulated surface 26.

As shown in Figure 8, the degassing valve is formed by first cutting a hole 31 out of the centre of one of the membranes, over which the valve will be placed. A washer 29 made of uncured silicone rubber is positioned around the hole on the smooth (outer) face of the membrane. A layer of hydrophobic PTFE membrane 30 with 0.2 micron pores and a thickness of 0.25mm is laid over the washer, and a second washer is placed on top. This is then repeated with a second PTFE membrane and a third washer.

When the bag is to be assembled, the two silicone rubber membranes are laid on top of one another, with the rough surfaces together. Two lengths if tubing for the inlet and outlet ports 23 are placed between the silicone membranes, protruding slightly into the rough area. The ports are provided with valves 24.

Next, room temperature vulcanising silicone rubber is applied to the untreated, smooth edges 27 of the silicone membranes, along which the membranes are to be joined to form a bag configuration. Uncured silicone rubber is applied around the tubing where it lies adjacent to the smooth edges of the membranes.

The constituents of the culture bag so arranged are then welded or glued together using elevated temperatures and pressure. The edges of the silicone membranes are sealed to form a bag, the degassing valve is formed from the layers of washers and PTFE membrane, and the tubing for the ports becomes integrated into the bag structure.

Figures 9 to 11 show the bio-reactor apparatus according to the fifth aspect of the invention. The apparatus is shown in Figure 9 comprising only two reactor tubes, whereas in practice a larger number, such as seven or eight tubes, is preferred. Each reactor tube 40

carries an internal coating of textured silicone rubber. To grow the cells on the interior surfaces of the tubes, the medium carrying the cell lines is introduced through an inlet 43. The reactor tubes are interconnected through the distributors 42. One or more strengthening members 45 ensure rigidity of the assembly. The assembly is rotated on rollers (not shown), followed by evacuation of the liquid and subsequent passage of nutrient medium over the cells. The medium is introduced through the inlet and issues from the outlet 44. The product is finally collected at the outlet.

As shown in Figure 10, the reactor tube comprises a non-porous silicone rubber tube 40 carrying an internal coating of textured silicone rubber 41, in accordance with the invention.

Figure 11 shows the reactor tubes, within which the dialysis tubes 51 are co-axially positioned. Cells are grown in the annular space 52 by the passage via introduction of medium comprising the cell line through the inlet 47. After removal of the liquid from the annular space through the outlet 48, the nutrient medium is passed through the dialysis tubes 51 via the medium inlet 49, issuing at the outlet 50. At the same time the liquor to undergo the bio-reaction is passed through the reactor tubes via the inlet 47, for collection at the outlet 48.

A microtitre plate according to the sixth aspect of the invention is shown in Figures 12 and 13. The standard type microtitre plate 60 has wells 61 without base walls. Either conventional microtitre plates are used and the base walls of the wells removed, or a microtitre plate is produced without any base walls. A non-porous silicone membrane 62 is attached to the bottom of the wells, the membrane comprises a silicone rubber sheet 63 having a coating with a textured surface 64 facing the area defined by the wells.

A tissue growth support structure according to the ninth aspect of the invention is illustrated in Figure 14. Tissue 83 is grown on pillars 81 of porous silicone rubber, the pores acting as a capillary system, supplying oxygen to the cells in the centre of the tissue mass. The pillars are attached to a gassing membrane 80 in a bio-reactor configuration, using gas permeable silicone rubber adhesive 82. The oxygen diffuses through the membrane and through the system of pores and channels to reach the tissue agglomerate.

In a preferred embodiment, HT-29 (intestinal carcinoma) cell tissue masses are grown on the artificial capillary system as shown. Experiments have shown that these tissue agglomerates can achieve a far greater size than when grown with no support.

Figure 15 shows an endocrine implant in accordance with the thirteenth aspect of the invention. Islet cells are immobilised within a bio-wafer 90 consisting of a disc of three dimensional porous silicone rubber 92, on which the islet cells are attached, sandwiched between semi-permeable layers 91, which allow the insulin out but stop the host from attaching and destroying the transplanted islet cells.

All of the above described aspects of the present invention depend upon the novel and inventive textured silicone rubber, with or without pores extending throughout the silicone structure, the textured surface being essential for cell anchorage and increased oxygen provision to the cells.

Claims

1. A method of making a silicone rubber having a structure adapted for growth of cells or living tissue, which comprises contacting a silicone rubber precursor with a biologically-acceptable sacrificial filler, curing the resultant mixture and removing the sacrificial filler to form a structured silicone rubber.

2. A method as claimed in claim 1, wherein the silicone rubber precursor can be cured or vulcanized at room temperature.

3. A method as claimed in claims 1 or 2, wherein the biologically-acceptable sacrificial filler is biocompatible, such that it is innately non-toxic and does not leave a toxic residue.

4. A method as claimed in claims 1, 2 or 3, wherein the sacrificial filler does not interact chemically with the silicone rubber precursor or with the resultant silicone rubber and is stable at temperatures used to cure the resultant mixture.

5. A method as claimed in any one of the preceding claims, wherein the sacrificial filler is granular and, preferably, crystalline.

6. A method as claimed in any one of claims 1-4, wherein the sacrificial filler is amorphous.

7. A method as claimed in any one of the preceding claims, wherein the sacrificial filler is ground and, preferably, classified, prior to contacting the silicone rubber precursor.

8. A method as claimed in claim 7, wherein the sacrificial filler is wet-milled, prior to mixing with the silicone rubber precursor.

9. A method as claimed in claims 7 or 8, wherein the sacrificial filler is milled to a particle size of 0.01-10 μm, preferably 0.05-1 μm, and most preferably 0.1-0.4 μm.

10. A method as claimed in claims 8 or 9, wherein the sacrificial filler is an inorganic salt and is milled in an organic solvent.

11. A method as claimed in any one of the preceding claims, wherein the sacrificial filler is an inorganic salt selected from the group consisting of metal halides, metal carbonates and metal bicarbonates.

12. A method as claimed in claim 11, wherein the inorganic salt is selected from the group consisting of lithium bicarbonate, sodium bicarbonate, potassium bicarbonate, lithium chloride, sodium chloride and potassium chloride.

13. A method as claimed in claim 12, wherein the sacrificial filler is sodium bicarbonate or sodium chloride, preferably food grade sodium bicarbonate or sodium chloride.

14. A method as claimed in claim 13, wherein the sodium bicarbonate or sodium chloride is wet-milled under xylene.

15. A method as claimed in any one of the preceding claims, wherein the sacrificial filler is removed by dissolution, preferably in an aqueous solvent.

16. A method as claimed in claim 15, wherein the sacrificial filler does not cause swelling of the silicone rubber when removed using an aqueous solvent.

17. A method as claimed in claim 16, wherein the sacrificial filler is sodium bicarbonate.

18. A method as claimed in any one of the preceding claims, wherein free -OH groups of the silicone rubber are chemically modified, so as to enhance cell adherence.

19. A method as claimed in any one of the preceding claims, wherein the surface of the silicone rubber is charged by bombardment with electrons.

20. A method as claimed in any one of the preceding claims, wherein the silicone rubber precursor comprises at least one additive that is not removed with the sacrificial filler and serves to impart desired physical properties on the rubber.

21. A method as claimed in claim 20, wherein the additive is a metal powder or carbon black and serves to render the silicone rubber electrically conductive.

22. A method as claimed in claim 21, wherein the additive is stainless steel powder.

23. A method as claimed in claim 21, wherein the additive is iron oxide.

24. A method as claimed in claim 20, wherein the additive is an inert substance, such as glass, and serves to render the silicone rubber mechanically rigid.

25. A method as claimed in any one of the preceding claims, wherein a surface of the silicone rubber precursor is contacted with the sacrificial filler, so as to form a structured silicone rubber having a textured surface.

26. A method as claimed in claim 25, wherein the textured surface of the silicone rubber facilitates attachment of adherent cells.

27. A method as claimed in claims 25 or 26, wherein the textured surface of the silicone rubber provides an increased number of sites for attachment of cells relative to an untextured surface.

28. A method of making a textured silicone rubber as claimed in claims 25, 26 or 27, which comprises forming a coating of a silicone rubber precursor on a substrate, contacting a surface of the coating with a biologically-acceptable sacrificial filler, curing the resultant mixture and removing the sacrificial filler to form a textured silicone rubber.

29. A method as claimed in claim 28, wherein the surface of the coating is contacted with the sacrificial filler under pressure, such that the sacrificial filler is substantially completely embedded in the coating.

30. A method as claimed in claim 29, wherein the sacrificial filler is embedded to a depth of 0.1-1.0 mm, preferably 0.1-0.5 mm, and most preferably 0.1-0.25 mm.

31. A method as claimed in claim 30, wherein the sacrificial filler is scattered or sprinkled over the surface of the coating, such that the sacrificial filler is only partially embedded in the surface.

32. A method as claimed in any one of claims 25-31, wherein the textured surface is micro-cupulated, the micro-cupules having a depth of less than 1 mm, preferably a depth of 0.5-0.1 mm.

33. A method as claimed in claim 32, wherein the micro-cupules measure less than 2 mm across, preferably less than 1 mm across, and, most preferably, less than 0.5 mm across.

34. A method as claimed in any one of claims 1-24, wherein the sacrificial filler is dispersed throughout the silicone rubber precursor, and the structured silicone rubber is substantially porous.

35. A method as claimed in claim 34, wherein the pores of the silicone rubber provide sites of attachment for cells or tissues, so that the cells or tissues may be substantially trapped within the resultant structure.

36. A method of making a porous silicone rubber as claimed in claims 34 or 35, which comprises mixing the biologically-acceptable sacrificial filler with the silicone rubber precursor, curing the resultant mixture at a temperature below 180°C, and removing the sacrificial filler, to form a porous silicone rubber.

37. A method as claimed in claims 34, 35 or 36, wherein the resultant mixture is shaped prior to curing, preferably by moulding or extrusion.

38. A method as claimed in any one of claims 34-37, wherein the pores are 1 μm-0.5 mm, preferably 10 μm to 0.2 mm, and most preferably 50 to 150 μm in diameter.

39. A method as claimed in any one of claims 34-38, wherein the porous silicone rubber is cut to a desired size or shape.

40. A method of making a textured or porous silicone rubber substantially as hereinbefore described with reference to the accompanying drawings.

41. A textured or porous silicone rubber obtained or obtainable by a method according to any one of the preceding claims.

42. A textured or porous silicone rubber substantially as hereinbefore described with reference to the accompanying drawings.

43. A biomedical device or apparatus comprising a textured or porous silicone rubber as claimed in claims 41 or 42.

44. A culture chamber for use in a method of culturing microbiological material, which comprises at least one gas-permeable wall or portion of a wall, and a textured interior growth surface arranged for contact with the microbiological material being cultured.

45. A culture chamber as claimed in claim 44, wherein the gas-permeable wall and the textured interior growth surface are each formed from an organic polymer.

46. A culture chamber as claimed in claim 45, wherein the gas-permeable wall and the textured interior growth surface are formed of the same organic polymer.

47. A culture chamber as claimed in claims 44, 45 or 46, wherein the at least one gas-permeable wall or portion of a wall also provides the textured interior growth surface.

48. A culture chamber as claimed in any one of claims 44-47, wherein the textured interior growth surface is a textured silicone rubber obtained or obtainable by a method according to any one of claims 5-17.

49. A culture chamber as claimed in any one of claims 44-48, wherein the at least one gas-permeable wall or portion of a wall is a silicone rubber membrane.

50. A culture chamber as claimed in any one of claims 44-49, including at least one port extending between the interior and the exterior of the chamber.

51. A culture chamber as claimed in claim 50, including an inlet port and an outlet port.

52. A culture chamber as claimed in claims 50 or 51, including at least one septum port.

53. A culture chamber as claimed in any one of claims 44-52, in the form of a flexible bag or envelope, preferably made of silicone rubber.

54. A culture chamber as claimed in any one of claims 44-53, including a valve means for release of gasses that build up within the chamber during use.

55. A culture chamber as claimed in claim 54, wherein the valve means comprises at least one filter means, the filter means allowing gasses to diffuse out of the chamber, but preventing microbial contamination thereof.

56. A culture chamber as claimed in claims 54 or 55, wherein the valve means comprises one or more layers of a hydrophobic porous material.

57. A culture chamber as claimed in claim 56, wherein the hydrophobic membrane is a PTFE membrane having a thickness of 0.25 mm and a porosity of 0.2 μm.

58. A culture chamber as claimed in any one of claims 44-57, further comprising a second chamber separated from the first chamber by means of a semi-permeable membrane.

59. A culture chamber as claimed in claim 58, wherein the second chamber has an access means separate from that of the first chamber.

60. An apparatus comprising a plurality of culture chambers as claimed in any of claims 44-59, for use in a method of culturing microbiological material.

61. An apparatus as claimed in claim 60, wherein the inlets of the culture chambers are interconnected and the outlets of the culture chambers are interconnected.

62. An apparatus as claimed in claims 60 or 61, wherein a further chamber having a semi-permeable wall is positioned within each culture chamber, each semi-permeable chamber having an inlet that is interconnected with the inlet of the other semi-permeable chambers and having an outlet that is interconnected with the outlet of the other semi-permeable chambers.

63. An apparatus as claimed in claim 62, wherein said apparatus is a bioreactor.

64. A method of culturing microbiological material in a culture chamber as claimed in any one of claims 44-59, or an apparatus as claimed in any one of claims 60-63.

65. A method as claimed in claim 64, comprising growing anchorage-dependent stromal cells on the textured surface of the culture chamber(s), and then inoculating anchorage-independent stem cells into the culture chamber(s), to allow proliferation of the stem cells.

66. A method of carrying out a bio-processing operation in a culture chamber or an apparatus as claimed in any one of claims 44-63, which comprises attaching cells for performing the bio-processing function to the textured surface of the culture chamber (s), introducing liquor to be processed into the culture chamber(s) via an inlet and collecting the processed liquor at an outlet from the culture chamber(s).

67. A method as claimed in claim 66, which comprises culturing cells on the textured surface in a culture medium, removing spent medium including cellular by-products from the culture chamber(s), and passing fresh nutrient medium through a semi-permeable chamber(s) located within the culture chamber(s), so to allow fresh medium to diffuse through the semi-permeable membrane into the culture chamber(s).

68. A method as claimed in claim 67, wherein the nutrient medium is passed through the semi-permeable chamber in the opposite direction to that in which the liquor or spent medium is passed through the culture chamber.

69. A method as claimed in claims 67 or 68, wherein the nutrient medium is recycled.

70. A well for use in a method of culturing microbiological material and having at least one wall defining the well, at least a portion of the wall being gas-permeable to enhance oxygen supply to the well, and at least a portion of the interior surface of the wall being textured to increase surface area and to enhance cell adherence.

71. A well as claimed in claim 70, wherein the gas-permeable portion of the wall and the textured portion of the wall are positioned at the base of the well.

72. A well as claimed in claims 70 or 71, wherein the gas-permeable portion of the wall comprises a gas-permeable membrane, preferably formed of silicone rubber.

73. A well as claimed in claims 71 and 72, wherein the membrane has a textured surface facing the interior of the well.

74. A well as claimed in any one of claims 70-73, wherein the textured surface has crater-like depressions or micro-cupules.

75. A well as claimed in any one of claims 70-74, wherein the textured surface is made by a method as claimed in any one of claims 25-33.

76. A microtitre plate having at least one well as claimed in any one of claims 70-75.

77. A method of culturing microbiological material on a well as claimed in any one of claims 70-75, or into a microtitre plate as claimed in claim 76.

78. An implant device comprising a cell support structure having a coating with a textured surface, to promote anchorage of the implant by cell attachment and ingrowth by surrounding tissue upon implant.

79. An implant device as claimed in claim 78, wherein the textured surface has crater-like depressions or micro-cupules.

80. An implant device as claimed in claims 78 or 79, wherein the coating comprises textured silicone rubber.

81. An implant device as claimed in claim 80, wherein the textured silicone rubber coating is made by a method as claimed in any one of claims 25-33.

82. An implant device as claimed in any one of claims 78-81, wherein the device is a heart valve, a sternum implant, or a reconstructed calf ligament.

83. A substrate for growth of skin grafts *in vitro*, comprising a flexible membrane having a textured surface.

84. A substrate as claimed in claim 83, wherein the flexible membrane prevents the skin graft from becoming brittle, whilst the textured surface increases the surface area for cell adhesion, promotes cell adhesion and gives the skin a rough surface to enhance "taking" of the graft on transplant.

85. A substrate as claimed in claims 83 or 84, wherein the flexible membrane is gas-permeable.

86. A substrate as claimed in claim 85, wherein the membrane comprises silicone rubber.

87. A substrate as claimed in any one of claims 84-86, wherein the textured surface has crater-like depressions or micro-cupules.

88. A substrate as claimed in any one of claims 84-87, wherein the textured surface is a textured silicone rubber made by a method as claimed in any one of claims 25-33.

89. A skin graft grown on a substrate as claimed in any one of claims 84-88.

90. A tissue support structure for use in a method of culturing tissue or cellular agglomerates, which comprises a biocompatible material having an internal system of pores, the pores promoting cell attachment and anchorage and oxygen supply to the tissue.

91. A tissue support structure as claimed in claim 90, wherein the porous material is provided with small, fine bore tubes.

92. A tissue support structure as claimed in claims 90 or 91, wherein the shape of the porous material is adapted so as to engineer the shape of the resultant tissue.

93. A tissue support structure as claimed in claim 90, wherein the porous material comprises porous silicone rubber.

94. A tissue support structure as claimed in claim 93, wherein the porous silicone rubber is made by a method as claimed in any one of claims 34-39.

95. An apparatus for culturing tissue or cellular agglomerates, comprising a tissue support structure as claimed in any one of claims 90-94, wherein the apparatus further comprises a gas-permeable membrane, to enhance oxygen supply to the system of pores and channels within the porous material, and therefore to the tissue.

96. An apparatus as claimed in claim 95, wherein the gas-permeable membrane is attached to the porous material.

97. An apparatus as claimed in claim 96, wherein the gas-permeable membrane comprises silicone rubber.

98. An apparatus as claimed in claims 96 or 97, wherein the porous material is attached to the gas-permeable membrane using a gas-permeable adhesive.

99. An apparatus as claimed in claim 98, wherein the gas-permeable adhesive is a silicone rubber adhesive.

100. An apparatus as claimed in any one of claims 94-99, wherein a plurality of tissue support structures are arranged in close proximity to one another, so as to allow fusion between tissue or cell masses growing on each structure, to create larger tissue or cellular agglomerates.

101. An artificial implant formed from a material having an internal system of pores, the pores promoting cell attachment and anchorage and oxygen supply to the cells on the implant surface.

102. An artificial implant as claimed in claim 101, wherein the porous material comprises porous silicone rubber.

103. An artificial implant as claimed in claim 101, wherein the porous material is made by a method as claimed in any one of claims 34-39.

104. An artificial implant as claimed in claims 101, 102, or 103, for use as a cartilage implant.

105. A cartilage implant as claimed in 104, wherein the porous material has been seeded *in vitro* with chondrocites, to form a layer of cartilage over the implant.

106. A cartilage implant as claimed in claims 104 or 105, for replacing eroded joints, wherein the porous silicone structure has been moulded to conform to the shape of the bone, which it is to protect.

107. A cartilage implant as claimed in claims 104, 105 or 106, wherein the porous material has been moulded into the shape of a nasal bridge.

108. A cartilage implant as claimed in claims 104, 105 or 106, wherein the porous material has been moulded into the shape of an ear.

109. An artificial implant as claimed in claims 101,102 or 103, for use as an artificial cornea.

110. An artificial cornea as claimed in claim 109, comprising a lens surrounded by an annular skirt formed of the porous material.

111. An artificial cornea as claimed in claim 110, wherein the porous material allows infiltration of the skirt by surrounding tissue after implantation, to hold the cornea in place.

112. An artificial cornea as claimed in claims 109, 110 or 111, wherein the lens is made from silicone rubber.

113. An artificial cornea as claimed in claim 112, wherein the lens is made from silicone rubber and the annular skirt is formed of porous silicone rubber, preferably made by a method as claimed in any one of claims 34-39.

114. An artificial implant as claimed in any one of claims 101, 102, or 103, for use as a vascular graft.

115. A vascular graft as claimed in claim 114, comprising a hollow tube made from porous material, preferably porous silicone rubber.

116. A vascular graft as claimed in claims 114 or 115, further providing an interior surface for cell adhesion.

117. A vascular graft as claimed in claim 116, wherein endothelial cells are grown on the interior surface of the graft.

118. A vascular graft as claimed in claims 116 or 117, providing an exterior surface for cell adhesion.

119. A vascular graft as claimed in claim 118, wherein smooth muscle cells are grown on the exterior surface of the graft.

120. A vascular graft as claimed in any one of claims 116-119, wherein one or both surfaces of the graft are additionally roughened to enhance cell attachment, preferably by providing the graft with textured silicone rubber surface.

121. A cell implant means comprising a porous material for retention of cells to be implanted, the pores promoting cell attachment and anchorage and oxygen supply to the cells, and a protective means to shield the cells from immune attack after implant.

122. A cell implant means as claimed in claim 121, wherein the porous material comprises silicone rubber.

123. A cell implant means as claimed in claim 122, wherein the porous silicone rubber is made by a method as claimed in any one of claims 34-39.

124. A cell implant means as claimed in claims 121, 122 or 123, wherein the protective means comprises a semi-permeable membrane forming an envelope around the porous material.

125. A cell implant means as claimed in any one of claims 121-124, for use as an endocrine implant.

126. An endocrine implant as claimed in claim 126, wherein the porous material is seeded *in vitro* with endocrine cells.

127. An endocrine implant as claimed in claim 127 wherein the endocrine cells are islets of Langerhans cells.

128. A drug delivery system comprising a porous material whose pores have been impregnated or saturated with a drug for delivery.

129. A drug delivery system as claimed in claims 128 or 129, suitable for implantation into a human or animal body.

130. A drug delivery system as claimed in claims 128, 129 or 130, wherein the drug is present in admixture with at least one sustained release ingredient.

131. A drug delivery system as claimed in claims 128, 129 or 130, wherein the porous material comprises a porous silicone rubber.

132. A drug delivery system as claimed in claim 131, wherein porous silicone rubber is made by a method as claimed in any one of claims 34-39.

133. A filtration media comprising porous silicone rubber, for use in separations.

134. A filtration media as claimed in claim 133, wherein the porous silicone rubber is made by a method as claimed in any one of claims 34-39.

135. A filtration media as claimed in claims 133 or 134, wherein the pores in the silicone rubber are of sub-micron size, preferably in the order of 0.1 – 0.5 μm.

136. A filtration media as claimed in claims 133, 134 or 135, for use in magnetic separation.

137. A filtration media as claimed in claim 136, wherein the porous silicone rubber includes magnetic additives.

138. A filtration media as claimed in any one of claims 133-137, for use in expanded bed absorption.

139. A filtration media as claimed in claim 138, wherein the porous silicone rubber is in particulate form.

140. A filtration media as claimed in any one of claims 133-139, for use in static inline filtration.

141. A filtration media as claimed in claim 140, wherein the porous silicone rubber is in the form of sheets or tubes.

142. A filtration media as claimed in any one of claims 133-141, wherein the porous silicone rubber is in the form or annular discs.

143. A cell cryopreservation system, comprising a porous material for absorbing cell culture into the internal system of pores and a container suitable for storage in liquid nitrogen.

144. A cell cryopreservation system as claimed in claim 143, wherein the porous material comprises porous silicone rubber.

145. A cell cryopreservation system as claimed in claim 144, wherein the porous silicone rubber is made by a method as claimed in any one of claims 34-39.

146. A cell cryopreservation system as claimed in claims 143, 144 or 145, wherein the container comprises releasable sealing means.

147. A cell cryopreservation system as claimed in claim 146, wherein the container is a syringe-type plunger.

148. An electrode comprising a porous material having electrically conductive particles dispersed therein.

149. An electrode as claimed in claim 148, wherein the porous material comprises porous silicone rubber.

150. An electrode as claimed in claim 149, wherein the porous silicone rubber is made by a method as claimed in any one of claims 34-39.

151. An electrode as claimed in claims 148, 149 or 150, wherein the conductive particles are metal or carbon powders.

152. An electrode as claimed in any one of claims 148-151, wherein the porosity of the material promotes adherence of microorganisms to the electrode surface, preferably microorganisms that are capable of digesting waste.

153. An electrode system comprising a plurality of electrodes as claimed in any one of claims 148-152 immersed in a liquid electrolyte and connected to an electric circuit.

154. A method of treating sewage using an electrode system as claimed in claim 153.

155. A wound dressing comprising a first layer of a porous gel and a second layer of a carrier gel.

156. A wound dressing as claimed in claim 155, wherein the porous gel layer comprises porous silicone rubber gel, preferably made by a method as claimed in any one of claims 34-39.

157. A wound dressing as claimed in claims 155 or 156, wherein the carrier gel layer comprises a silicone gel.

158. A wound dressing as claimed in claims 155, 156 or 157, wherein the carrier gel is applied to a supportive structure, preferably a Dacron® mesh.

159. A wound dressing as claimed in any one of claims 155-158, wherein the porous gel layer is infused with a drug for delivery to the wound.

160. A wound dressing as claimed in claim 159, wherein the drug is a growth-promoting drug.

161. A wound dressing as claimed in claim 159, wherein the drug is an antibiotic.

162. A clinical swab, comprising a porous material, the pores increasing the surface area of the swab and promoting oxygen transport to the swab surface.

163. A clinical swab as claimed in claim 162, wherein the porous material comprises porous silicone rubber.

164. A clinical swab as claimed in claim 163, wherein the porous silicone rubber is made by a method as claimed in any one of claims 34-39.

165. A clinical swab as claimed in claims 162, 163 or 164, wherein the porous material contains a radio-opaque additive, preferably barium sulphate.

166. A clinical swab as claimed in any one of claims 162-165, wherein the porous material is infused with a drug.

Abstract

A method of making a silicone rubber having a structure adapted for growth of cells or living tissue, which comprises contacting a silicone rubber precursor with a biologically-acceptable sacrificial filler, curing the resultant mixture and removing the sacrificial filler to form a structured silicone rubber.

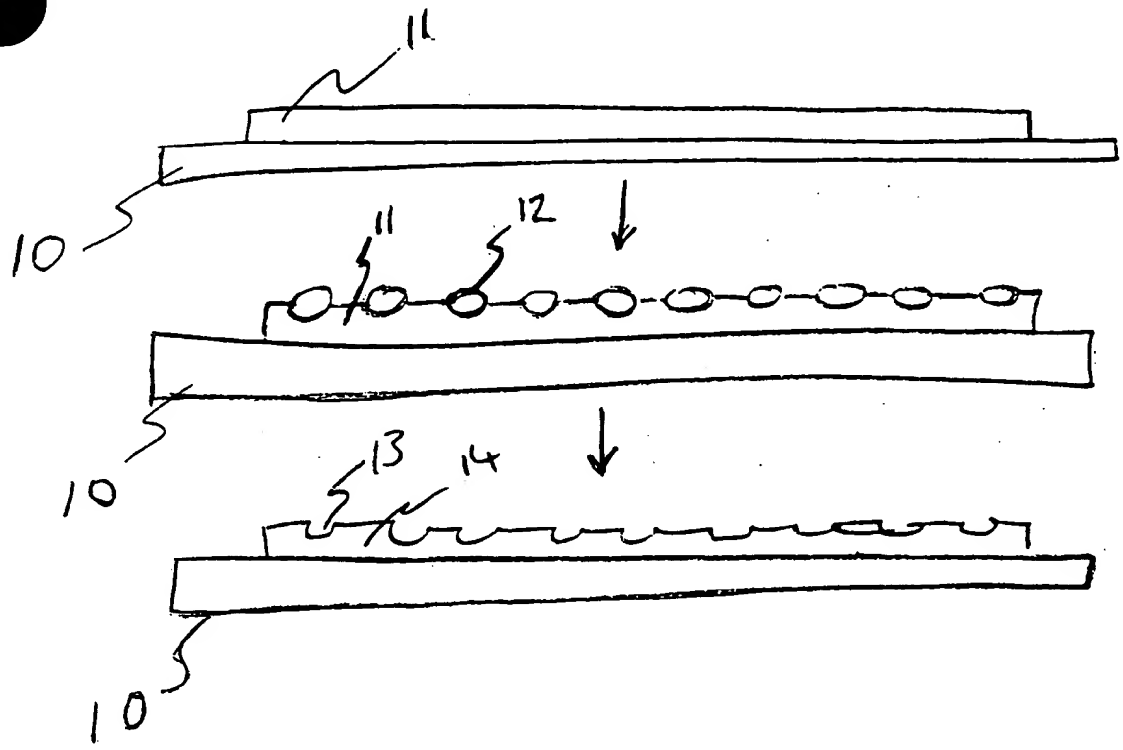
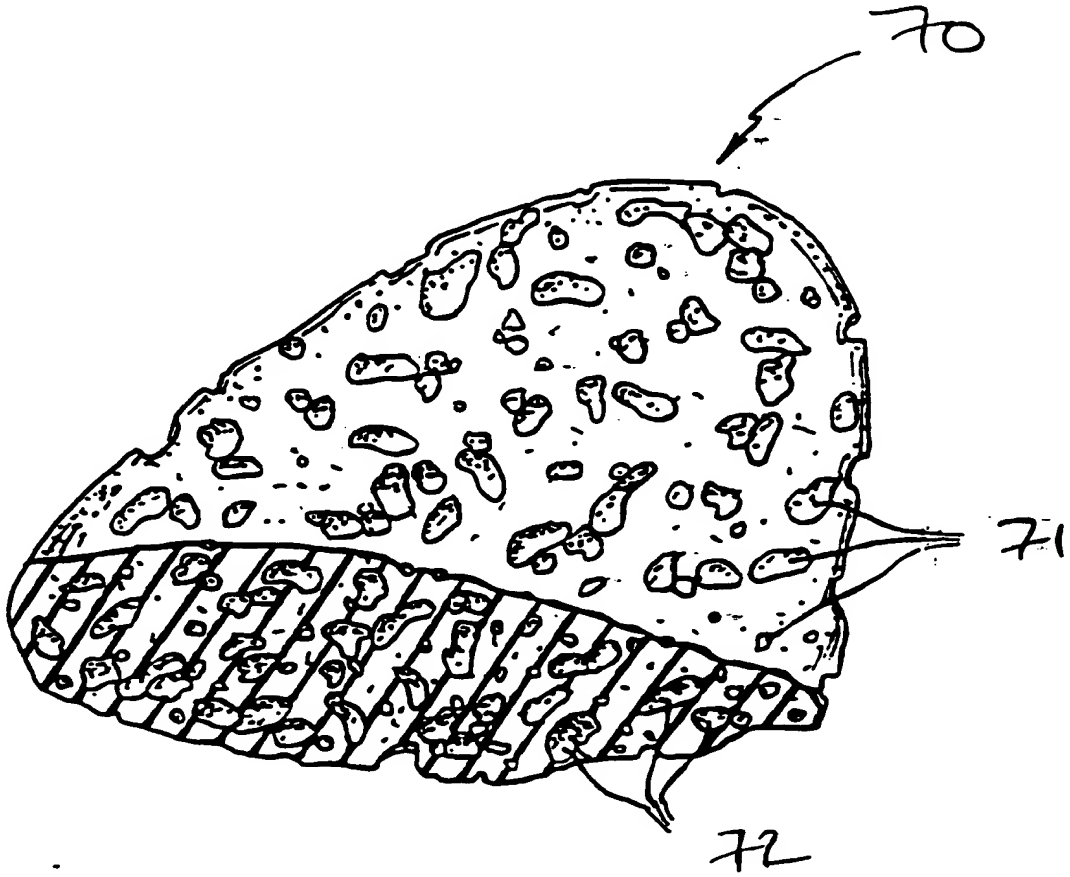


Fig 1

Fig 2

Fig 3

Fig. 4



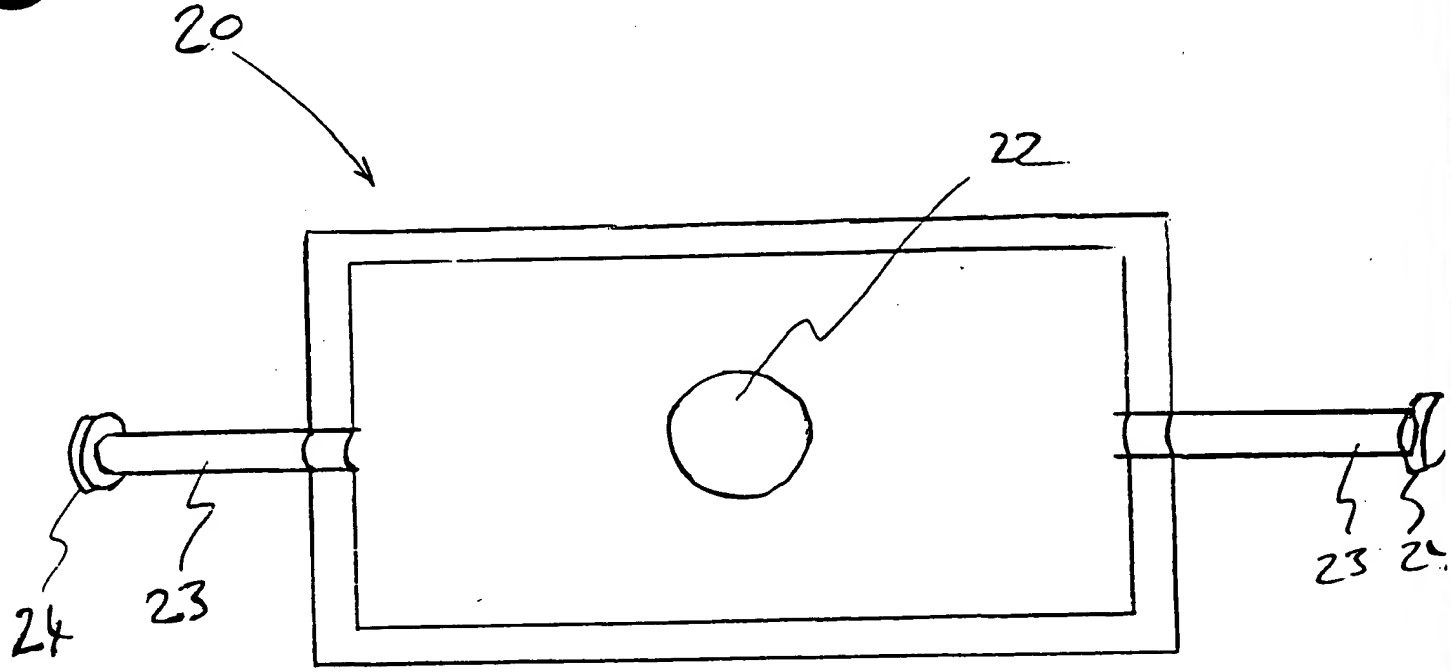


Fig. 5

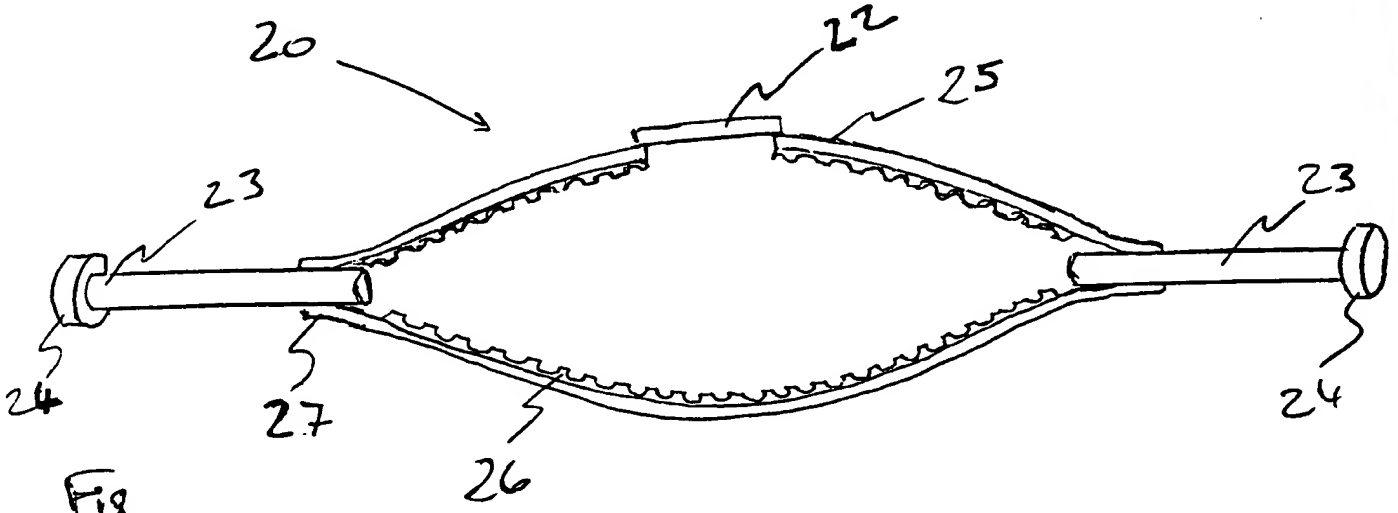


Fig. 6

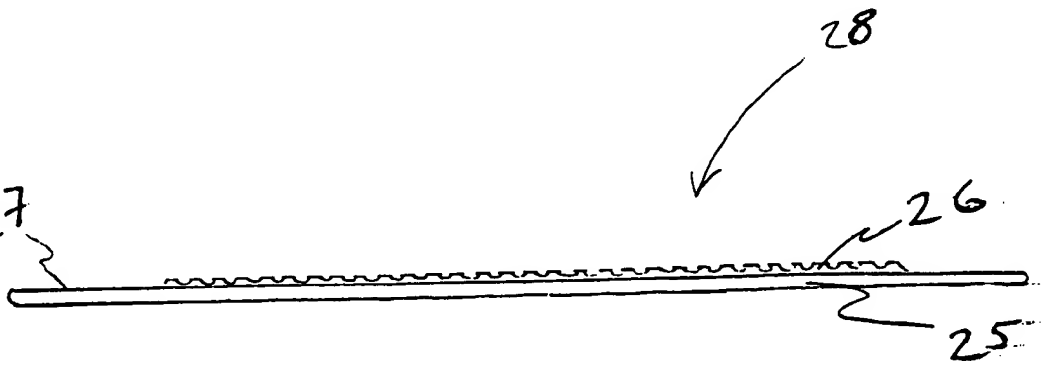


Fig. 7

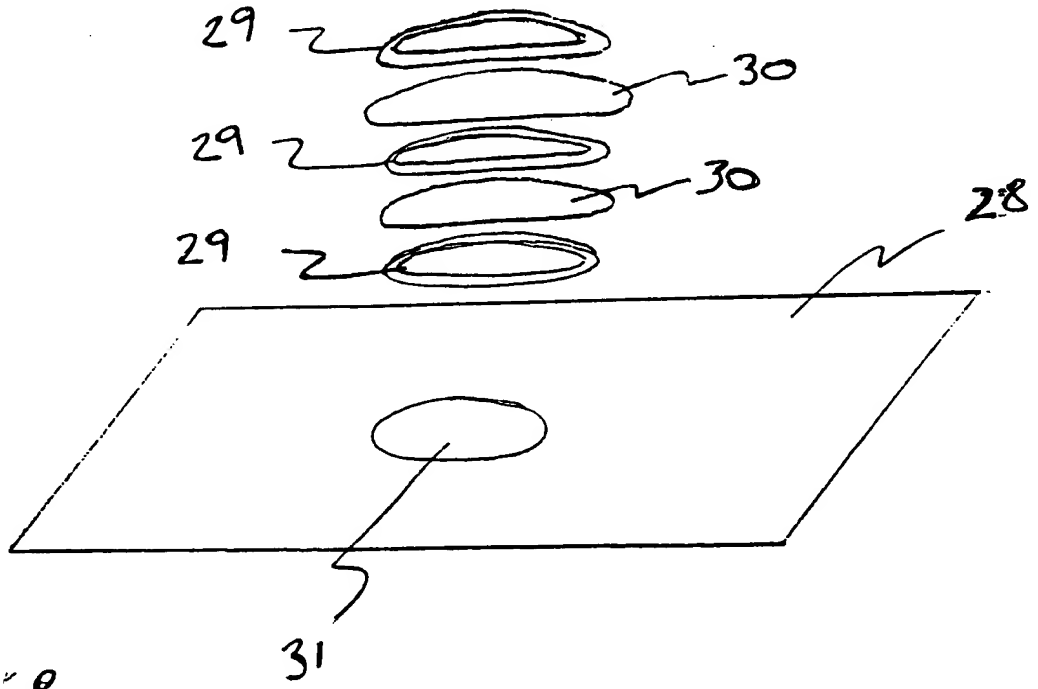


Fig. 8

Figure 9

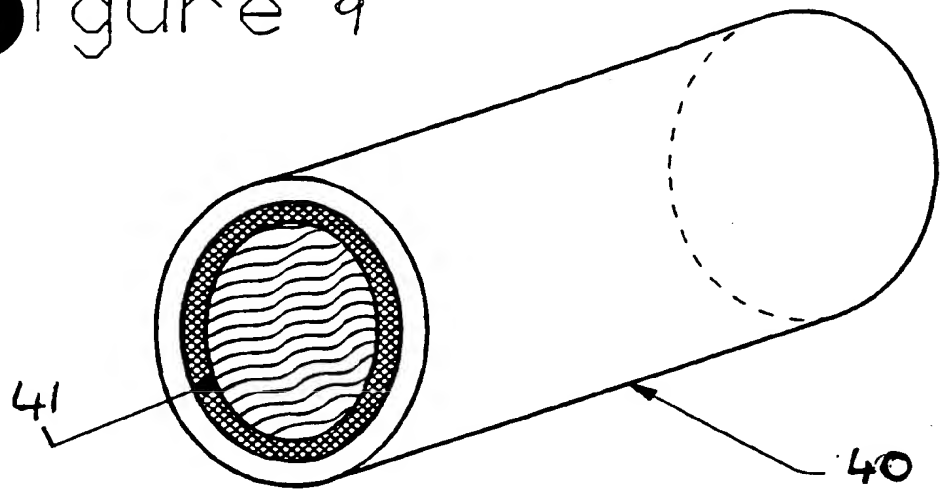


Figure 10

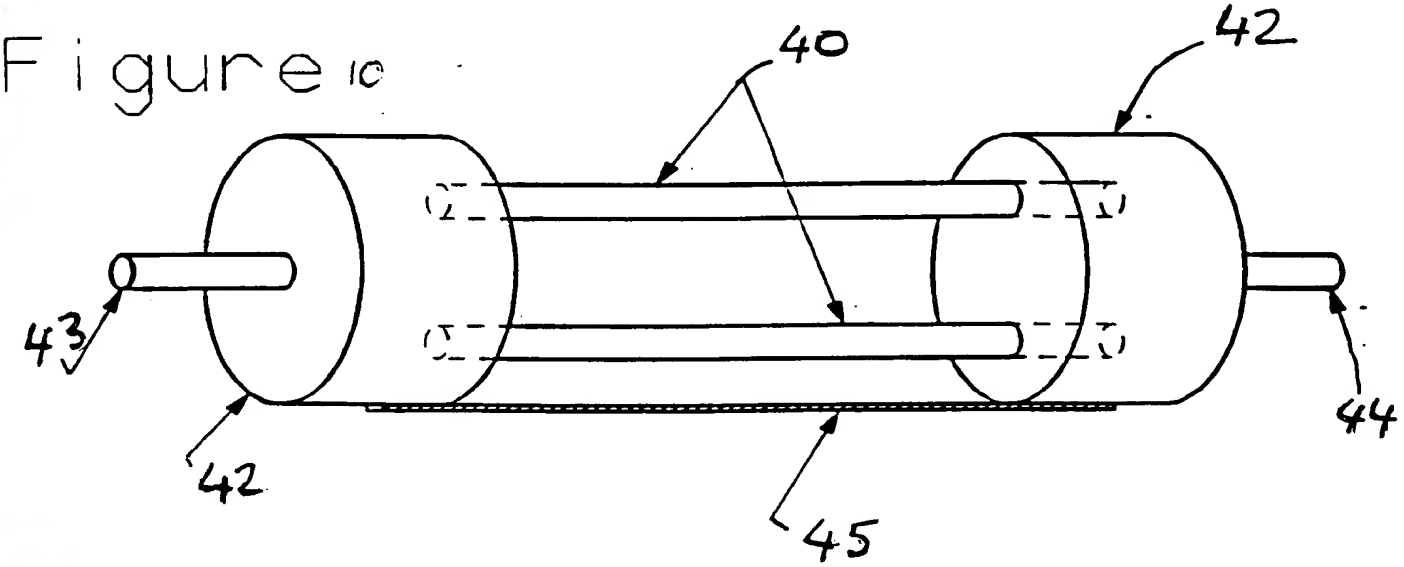
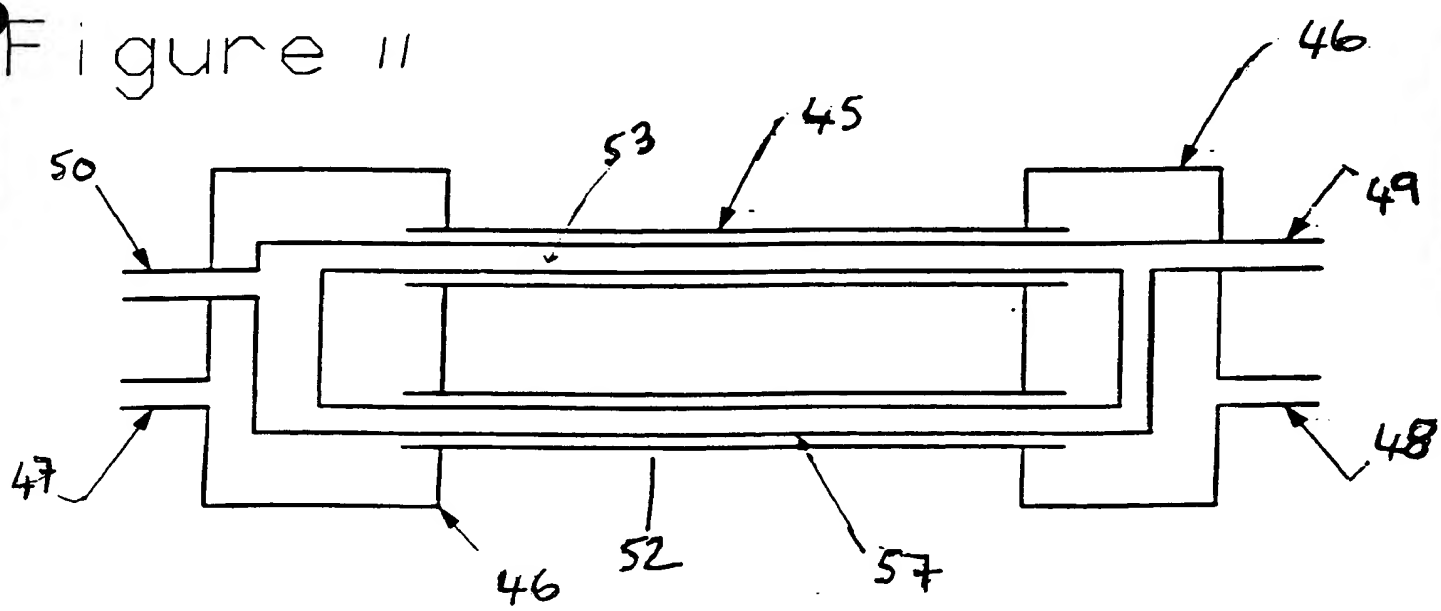


Figure 11



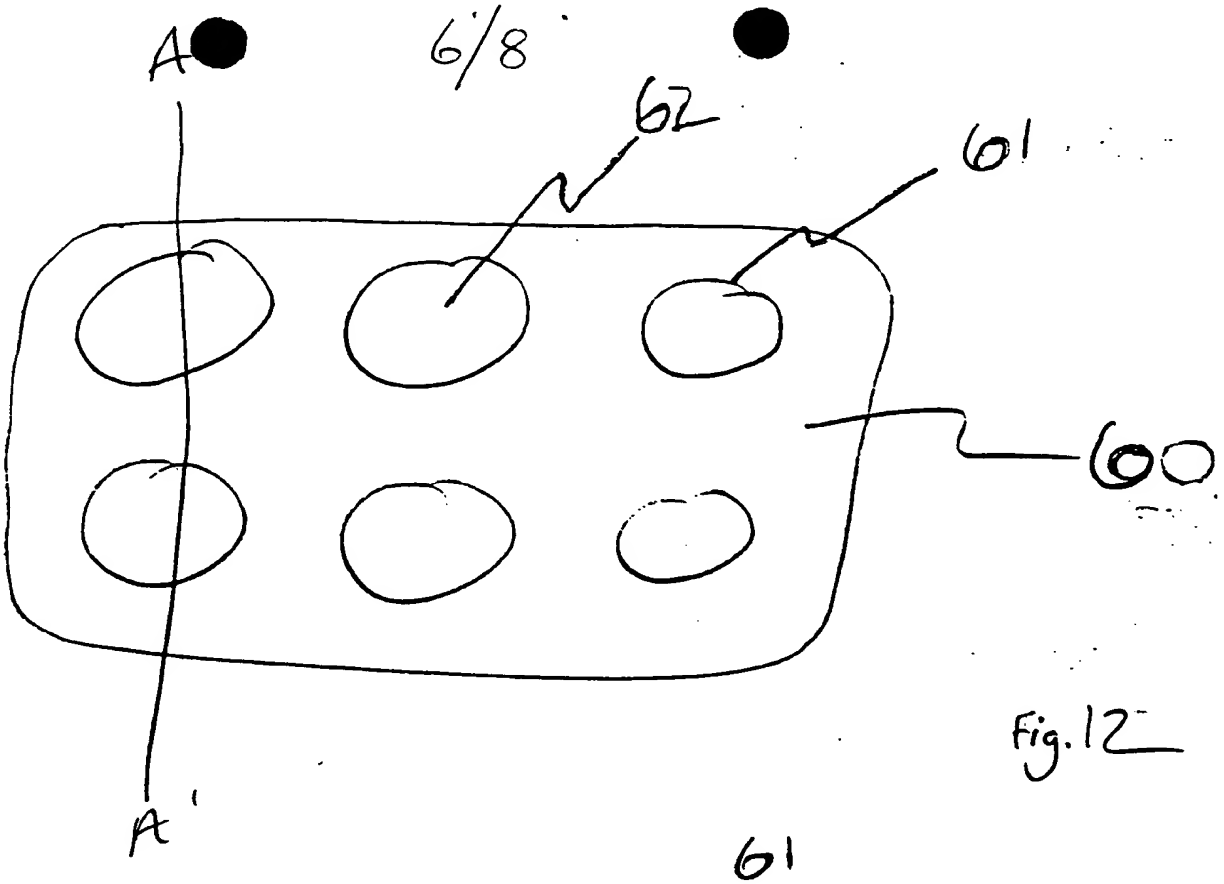


Fig. 12

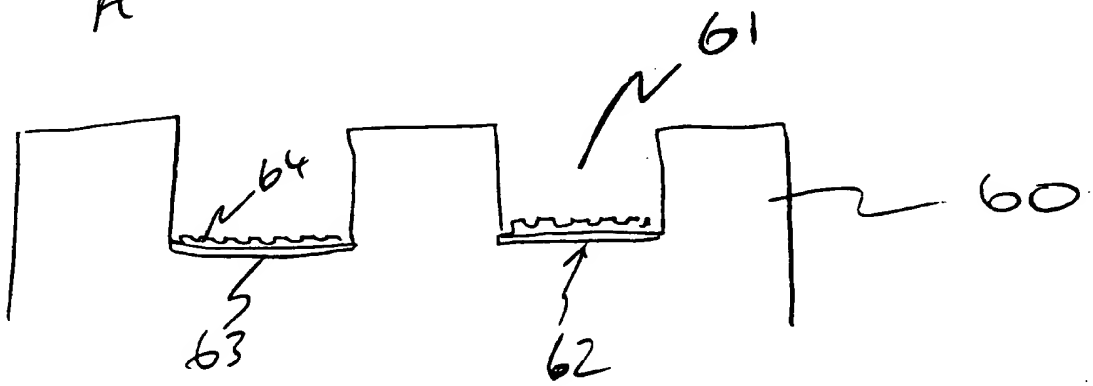


Fig. 13

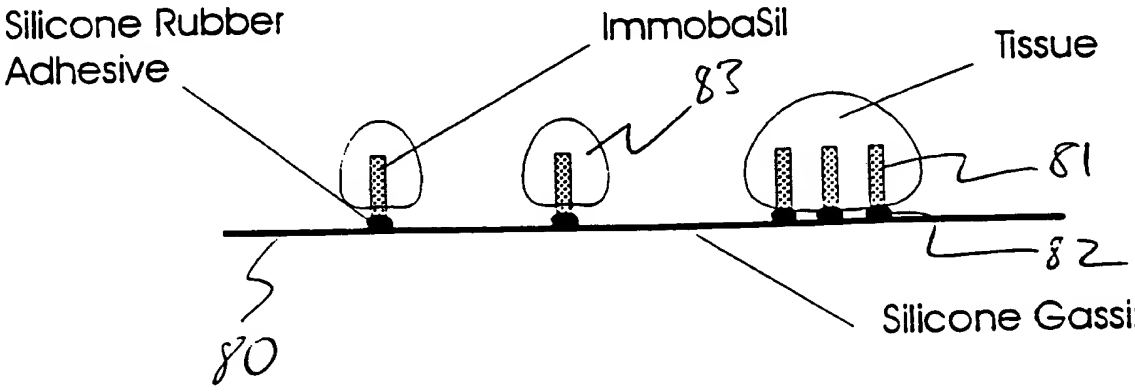

Silicone Rubber
Adhesive
ImmobaSil
83
Tissue
81
82
80
Silicone Gassing Membran

Fig 14

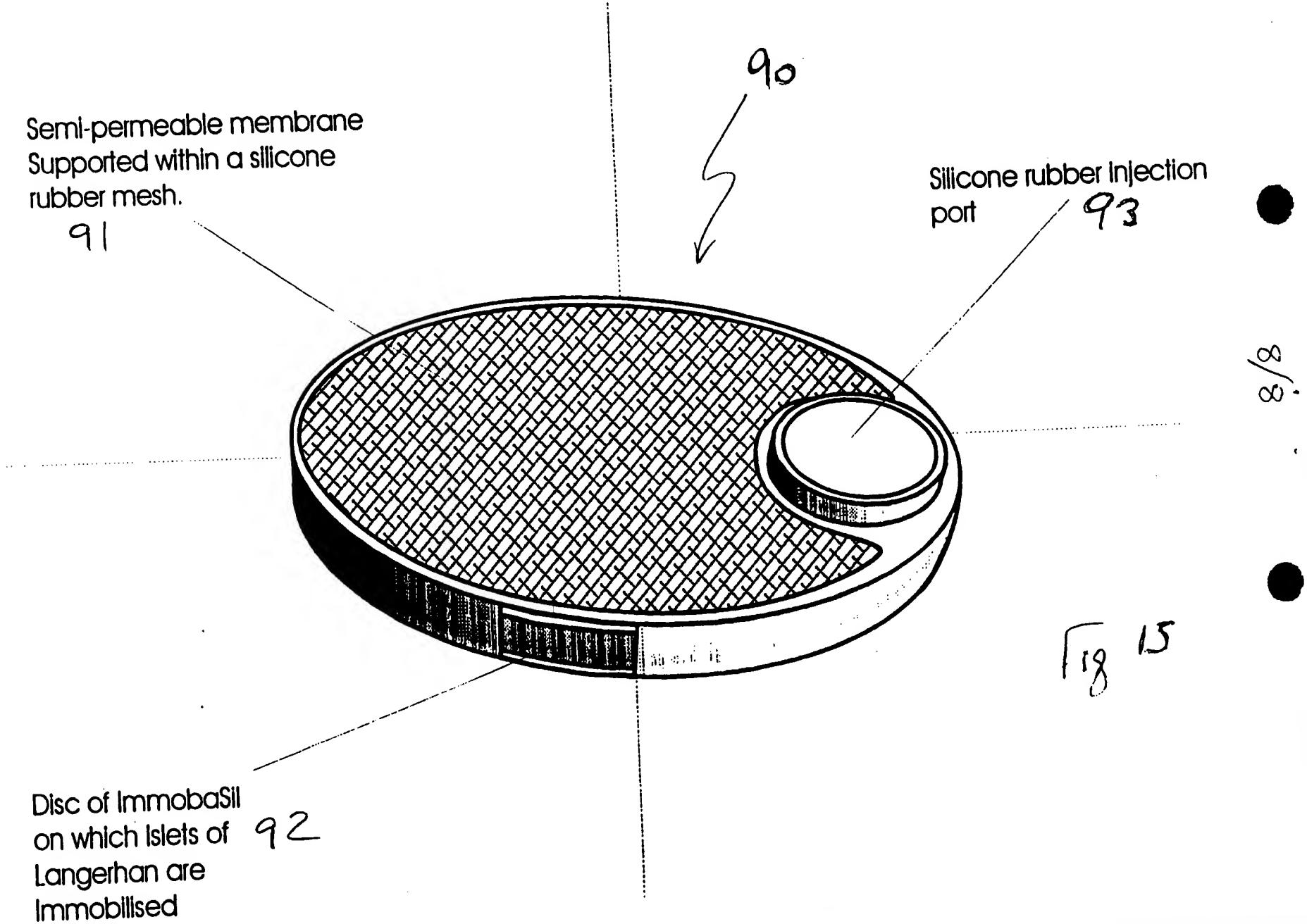

90
Semi-permeable membrane Supported within a silicone rubber mesh.
91
Silicone rubber injection port
93
Disc of ImmobaSil on which Islets of Langerhan are Immobilised
92

Fig 15

[illegible]

[illegible]

[illegible]